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A General Method for the Determination of Mercury in the Various Types of Pharmaceutical Preparations*

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The United States Pharmacopœia XII and the National Formulary VII contain some thirty-six compounds or preparations in which mercury is a component and recommend seven more or less distinct methods for the determination of this element. The inclusion of the Book of Methods of the A. O. A. C. and the New and Non-official Remedies increases the number of procedures to about twelve. There are a great number of mercury-containing preparations which are not listed in the above volumes and for which no assay procedure is recommended. The analyst in dealing with the latter is faced with the necessity of choosing one of the twelve procedures which would appear to be most applicable. It seemed that the confusion arising from this plethora of methods for the determination of mercury might be eliminated by the development of a simple generally applicable procedure.

Bouton and Duschak (1) reported a rapid volatilization procedure especially developed for mercury ores. They included a rather complete bibliography of the various methods developed for the determination of mercury. Garratt (2) has summarized the various procedures currently employed for the products in the British Pharmaceutical Codex. Jorgensen (3) reviewed the results of the work done by the referees and collaborators for the A. O. A. C. during the past several decades. Fitzgibbon (4) and Sloviter, McNabb and Wagner (5) have given more detailed reviews of procedures based on the Rupp iodine method.

The starting point of the method here reported was the ethanolamine reduction procedure reported by Rauscher (6). A very desirable feature of the ethanolamine procedure was the fact that the reduction of the mercury to the metallic state made possible its separation from organic matter and halides, thus permitting the titration of the redissolved metal with thiocyanate and thereby effecting a considerable saving in time. However, in applying the method to the assay of commercial preparations, it was soon found that difficulty was experienced in obtaining consistent results. Fine globules of mercury often remained floating on the surface of the ethanolamine and were lost in the decantation operation. Shukis and Tallman (7) attempted to overcome this by centrifuging the reduced solution before decanting the supernatant solution. This modification did not completely solve the difficulty since many organic substances and salts normally mixed in the usual pharmaceutical preparations prevent ag-

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glomeration of the mercury into discrete globules. The more or less colloidal suspension of finely divided metallic mercury could not be consistently recovered quantitatively.

Early in the application of the method it was noticed that much more consistent results were obtained when Rausher's method II was used. The better results were attributed to the amalgamation of the excess sodium in the ethanolamine with the mercury to form a solid pellet, which could be separated from the reducing solution without difficulty. In order to obviate the use of metallic sodium, and yet retain the advantage of amalgamation, powdered zinc was tried. It was found that the use of zinc, aside from its economy, was more practical in that the resulting amalgam did not decompose on washing with alcohol and water. Zinc in alkaline, neutral or acid solutions has long been employed in reducing reactions. The ability of zinc, as well as other relatively electronegative elements, to displace the more "noble" mercury, silver, gold, etc., from compounds is frequently used to advantage in analytical procedures. Recently Winkler (8, 9), Waterhouse (10), Read and Maize (11), Ferrey (12), Pierce (13) and Chambers (14) reported the use of zinc while Eckert (15) used aluminum for the purpose of displacing mercury in various types of mercury preparations. The separation of the zinc-amalgam from the reducing solution is more completely effected by filtration with the aid of slight suction through a well-packed pad of asbestos pulp. The excess zinc powder suspended in the solution forms a layer on the surface of the asbestos pad which serves to prevent any ionic mercury from passing into the filtrate.

In the practical application of the method it was found that the efficiency of the reducing mixture was materially increased by the addition of a few millimeter of a 1:1 solution of caustic alkali. This serves to change a number of the volatile mercurials into less volatile complexes which remain in the solution where they can be acted upon by the reducing mixture. The nascent hydrogen evolved by the solution of some of the zinc in the alkali adds to the reducing power of the mixture. In many cases it was found that "practical" triethanolamine was superior to either mono- or diethanolamine. Likewise, the variety of ingredients mixed with the mercurial in the numerous preparations often requires the aid of some solvent other than dioxane. In many cases the higher boiling alcohols or glycols and/or xylene or toluene were found to be a distinct aid in the reduction mixture. Similarly, benzene, petroleum ether, ethyl ether and alcohol were found advantageous in washing the amalgam free of organic substances often admixed with the mercurial. Frequently, the presence of materials which form gelatinous hydrates or metallic soaps render filtration almost impossible without the aid of acetic acid.

EXPERIMENTAL

The procedure described below has been found applicable to a large number of comparatively pure mercurials as well as samples of commercial preparations including tinctures, solutions, ointments, powders and tablets. Minor variations dictated by the experience and judgment of the analyst which will facilitate the handling of specific samples do not affect the accuracy of the method.

Apparatus.—A round-bottom flask of about 100–125 ml. capacity with a standard taper neck.

A straight tube (Liebig) type water-jacketed reflux condenser with a ground glass joint to fit the flask.

A small filtering tube or crucible with a removable porcelain or sintered glass disk of about 15 mm. diameter.

A suction flask assembly to accommodate the filter tube.

Burettes, 5 ml. graduated in 0.01 ml. and 10 ml. graduated in 0.05 ml.

Reagents.—Mono-, di- or (preferably) triethanolamine. (Eastman's "practical" or equivalent grade has been found satisfactory.)

Zinc powder or dust, reagent quality.

Solvents—dioxane, acetone, alcohols, acetic acid, xylene, toluene, petroleum ether, etc., of usual analytical grade, and nitric acid, reagent quality and halide free.

Potassium permanganate, 5% solution.

Dilute hydrogen peroxide.

Ferric ammonium sulfate, 10% solution (U. S. P. Test Solution).

Standardized solutions of ammonium thiocyanate, 0.02 *N* and 0.05 *N*.

Asbestos pulp, finely cut and digested in dilute alkali, then in nitric acid and thoroughly washed.

Procedure.—Place the sample calculated to yield from 2 to 50 mg. of mercury in the flask and add 10 ml. of a mixture consisting of two volumes of ethanolamine, 2 volumes of xylene or toluene, and 1 volume of butyl alcohol. Add 2–3 ml. of strong ammonia or of a 50% solution of sodium or potassium hydroxide, followed by 0.2–0.3 Gm. powdered zinc. Connect the flask to the reflux condenser, place on an asbestos board with a hole cut in its center to fit the tip of the flask and heat over a small flame. A boiling period of five to fifteen minutes is sufficient to complete the reduction of most inorganic salts in fairly concentrated forms. However, many organic mercurials or preparations with a large proportion of diluents usually require a boiling period of about thirty minutes. The end of the reduction is usually marked by a clear layer of supernatant solution and a solid residue of mossy zinc amalgam together with the excess zinc powder. Occasionally, the presence of substances like fatty ingredients in some ointments causes the formation of a suspension of powdered zinc in a more or less viscous gelatinous emulsion. This condition is easily overcome by cooling the flask somewhat and cautiously adding 3–5 ml. of glacial acetic acid through the top of the condenser. A few minutes' additional boiling will then break the emulsion and cause the agglomeration of mossy-zinc amalgam.

Allow the flask to cool to about 50° and wash down the condenser with a few ml. of alcohol, disconnect the condenser and rinse the tip of the condenser and neck of the flask with a little more alcohol, collecting all rinsings in the flask. Pour the supernatant solution into the filtering tube prepared with a well-packed pad of asbestos about 7–8 mm. thick, retaining as much of the zinc amalgam in the flask as possible. Wash the flask and the amalgam free of reducing solution and organic matter with any appropriate solvent. Benzene, petroleum ether, etc., may be required to remove the base of some ointments. Wash out the organic solvent with several small portions of alcohol or acetone and three or four small portions of water, pouring all washings through the filter pad.

Transfer the asbestos pad and any metallic residue back to the flask containing the bulk of the amalgam. Add 3–5 ml. of water and place the flask under a condenser, previously well washed to remove traces of halides. Add slowly through the top of the condenser 6–8 ml. of concentrated nitric acid (halide free). After the initial reaction subsides, heat the flask and boil two to three minutes to dissolve the amalgam and to drive out most of the oxides of nitrogen. Cool the flask somewhat and inspect the flask for any small globules of undissolved mercury in the suspension of asbestos. Allow the flask to cool to about 70° and pour down the condenser several small portions of water, disconnect the condenser and rinse its tip and the neck of the flask with more water. While the solution in the flask is still hot add a few drops of potassium permanganate so that a deep purple color remains on cooling the flask to about 20°. Dilute the solution to 80–90 ml. and discharge the permanganate color with a drop of hydrogen peroxide. Add 2 ml. of ferric alum indicator solution and titrate with standard ammonium thiocyanate solution without undue delay.

1 ml. 0.02*N*·NH_4SCN = 0.0020 Gm. Hg

DISCUSSION

It is well to bear in mind that while the presence of halides aids the reduction of the mercury, it is essential that they be washed out completely before titration with thiocyanate. Any appreciable amount of organic matter or lumps of amalgam may occlude sufficient halides to seriously vitiate the thiocyanate titration. Bouton and Duschak (1) have shown that minute traces of halides cause a disproportionate error in the final result. Therefore, the use of any solvent which will dissolve and wash out the organic matter and of a glass rod to break up any lumps of amalgam is desirable. Usually, 2 or 3 washings with organic solvents followed by several alcohol or acetone washings and finally 3 or 4 rinsings with distilled water effect the complete removal of the halides.

The preparation of the samples for assay determines, in a large measure, the success of any procedure. The large number and variety of mercury preparations studied during the past six years resulted in the following more or less general methods of preparing the various types of samples for assay by the method described in preceding paragraphs.

I. *Mercury Salts and Organic Mercurials.*—Those products containing little or no foreign matter have been found to be more easily handled by weighing 5–10 times the desired amount for assay and dissolving it with any appropriate solvent. The solution of the sample is then diluted to a definite volume in standard volumetric flask. In most cases the addition of 10–15 ml. of approximately normal potassium bromide-bromate solution (3 Gm. $KBrO_3$ and 12 Gm. KBr in 100 ml. water) followed by 3–5 ml. of concentrated hydrochloric acid quickly and effectively aids the solution of the sample. A few organic compounds which form water-insoluble brominated products may require the addition of a little glycerin and/or alcohol, dioxane, etc., for solution. Iodine and chlorine solutions have been used for the same purpose but bromine has been found to be more generally applicable and easier to handle. Ordinarily the heat of solution produced by the addition of the hydrochloric acid is sufficient quickly to decompose most of the inorganic salts. Organic mercurials, however, may require a three- to five-minute digestion on a steam bath. In the latter case, the digestion is best carried out in a small flask with a glass joint to fit a water-cooled condenser. After allowing the digested sample to cool, the condenser is washed down with a little water and the tip of the condenser and neck of the flask carefully rinsed. The cooled solution, washings and rinsings are then transferred to a volumetric flask and diluted to the mark. Suitable aliquots are then transferred to the reduction flask, and the solution made slightly alkaline by the addition of pellets of potassium hydroxide. The residual bromine quickly disappears on the addition of the powdered zinc and ethanolamine solution.

The treatment with the halide aids materially in converting the mercury to an ionic form which enables the reducing reaction to go to completion in a much shorter time. This is especially the case with those preparations which contain sulfur. A five- to ten-minute digestion (reflux) on a steam bath produces solutions which are easily assayed. Without the halide treatment mercury-sulfide complexes would be formed in the reduction mixture which make the quantitative determination of mercury almost impossible. Sample No. 14 in Table I shows the effectiveness of this procedure. The mercury sulfide was obtained by treating a sample (No. 19) of merthiolate essentially as outlined by the N. N. R. (18) method. The sulfide was then filtered, washed, dried and weighed in a manner similar to the micro-method of Pregl for halides (19). The washing of the sulfide included several small portions of warm benzene which aids in dissolving difficultly soluble sulfur complexes that usually accompany mercury sulfide when it is precipitated in the presence of organic solvents. Chambers (14) reported that benzene with or without the aid of acetic acid serves to prevent high results in the gravimetric sulfide methods. After completion of the gravimetric determination, the asbestos mat and sulfide were transferred quantitatively from the filter tube to the reducing flask, digested with bromine solution until all of the black specks of sulfide disappeared, the solution cooled and the assay completed as outlined above. The results indicate complete recovery.

II. *Solutions and Tinctures of Mercurials.*—These products would seem to be beyond the difficulty of representative sampling. However, it has been found that many such preparations "age" and change appreciably due to the ease with which many substances reduce mercury to a state whereby an ionic exchange takes place and permits the deposition of mercury on the walls of the container. Up-

wards of 50% of the mercury in many of the more dilute solutions and tinctures is often apparently lost and can be recovered from the walls of the container. See Table II.

Samples of liquid preparations containing mercury equivalent to 50 mg. or more per ml. are best handled by taking 5–10 times the desired amount, digesting with bromine if necessary, and diluting in a standard volumetric flask. Aliquots yielding 10–50 mg. of mercury are then taken for assay.

In order to increase the speed and effectiveness of the reduction reaction it has been found desirable to keep the volume of the sample solution to about 10 ml. Very dilute solutions requiring upwards of 25 ml. of sample to yield 2–10 mg. of mercury are better handled by taking sufficient sample to yield 0.5 mg. or less of mercury and reducing the mercury as above. However, instead of titrating the nitric acid solution of the zinc amalgam with thiocyanate, it is filtered clear of the suspended asbestos fiber, and the filtrate diluted to a standard volume in a volumetric flask. Convenient aliquots of the solution are then titrated by the dithizone procedure as given in the "Book of Methods" (16) or by the modification which has been suggested by Laug and Nelson (17).

III. *Ointments.*—Since it is almost impossible to prevent a certain amount of segregation in mechanical mixtures, large differences often occur in the mercury content of samples of ointments taken from the various parts of the container. Consequently, it is necessary to transfer the ointment to a dish and thoroughly mix it before any attempt is made to

TABLE I.—INORGANIC AND ORGANIC MERCURIALS

Sample No.	Mercurial	Sample Taken, Mg.	Mercury Found, Mg.	Mercury Found, %	Mercury Calculated, %
1	Mercury Ammoniated	53.3	42.0	78.6	78.0–80.0
2	Mercury Ammoniated	16.0	12.5	78.5	78.0–80.0
3	Mercury Ammoniated[a]	80.0	63.0	78.8	78.0–80.0
4	Mercury Ammoniated[b]	80.0	63.1	78.9	78.0–80.0
5	Mercuric Chloride	10.2	7.5	73.5	73.5–73.9
6	Mercurous Chloride	20.0	16.9	84.5	84.7–85.0
7	Mercurous Chloride	26.3	22.1	84.0	84.7–85.0
8	Mercurous Chloride[b]	26.3	22.2	84.3	84.7–85.0
9	Mercuric Cyanide	49.1	37.9	76.3	79.4(?)
10	Mercuric Iodide	18.4	8.2	44.2	44.5
11	Mercuric Succinate	42.1	25.5	60.5	61.0
12	Mercuric Succinimide	43.6	22.1	50.7	50.6
13	Mercuric Sulfate	59.4	40.0	67.0	67.6
14	Mercuric Sulfide[c]	33.0	28.4	86.0	86.2
15	Merbromin	51.1	12.9	25.3	24.0–26.7
16	Meroxyl	44.8	11.3	25.3	26.0–29
17	Merthiolate	53.8	26.2	48.6	49.15–49.65
18	Merthiolate	58.0	28.3	48.9	49.15–49.65
19	Merthiolate[b]	58.0	28.4	49.0	49.15–49.65
20	Mercresin	42.9	25.8	60.0	61.0
21	Metaphen	54.9	31.4	57.1	57.0
22	Phenyl Mercuric Acetate	44.3	26.3	59.3	59.6
23	Phenyl Mercuric Nitrate	33.1	21.4	64.5	64.5
24	Phenyl Mercuric Picrate	28.5	11.1	39.0	40.0(?)
25	Flumerin	33.8	10.8	32.2	33.8
26	Furyl Mercuri Hydroxide	24.0	17.1	71.3	70.5
27	Furyl Mercuri Chloride	26.8	17.7	66.0	66.0

[a] Precipitate of mercury as $HgZn(SCN)_4$ titrated with KIO_3.
[b] Precipitation as mercuric sulfide (gravimetric determination).
[c] Mercuric sulfide obtained from Merthiolate sample No. 19.

TABLE II.—LIQUID PREPARATIONS

Sample No.	Preparation	Sample, Ml.	Mercury, Mg./Ml. Theory	Mercury, Mg./Ml. Label Claim	Mercury, Mg./Ml. Found	Recovery, %
1	Merbaphen (Novasurol)	0.4	...	33.0–34.5	34.7	...
2	Merbromin[a] (Surgical)	2.0	51.4	....	51.3	99.8
3	Mercupurin	0.8	...	37.0–42.0	38.0	...
4	Mercupurin[a]	1.0	38.5	38.5	...	100.0
5	*o*-Hydroxyphenyl-HgCl[a]	10.0	6.3	....	6.2	98.4
6	Mercresin	10.0	...	0.57 (1:1000)	0.61	...
7	Merthiolate	10.0	...	0.495 (1:1000)	0.49	...
8	Merthiolate[a]	10.0	0.486	....	0.49	100.8
9	Metaphen	2.0	...	2.8–2.85 (1:200)	2.8	...
10	Metaphen[a]	2.0	2.85	....	2.85	100.0
11	Merphenyl-picrate[a]	10.0	0.39	....	0.385	98.7

[a] Sample solutions made from previously assayed samples of the mercurial to which were added substances to simulate the commercial products.

weigh out samples. A convenient method of accurately weighing the desired amount of sample is to fill the well-mixed ointment into a clean, plastic collapsible tube and squeeze the sample directly into the reducing flask. Another expedient is to draw the end of a 10–12 mm. O.D. glass tube to 1–2 mm. and cut the tube to a length of 6–8 cm. A plunger can be made from a glass rod which fits snugly into the tube. Such simple improvised syringes save much time in the weighing and transferring of ointment samples.

Usually 50–100 mg. of ointment sample contains ample mercury for easy and accurate assay. The ethanolamine solution, alkali and zinc powder are added directly to the sample and the assay completed as outlined. While as much as 5 Gm. of ointment has been successfully assayed it is not advisable to work with samples in excess of 2 Gm. If more sample is required to yield 2–10 mg. of mercury it is better to reduce the sample so that not over 0.5 mg. of mercury is obtained in the amalgam and make the final determinations by the dithizone procedure. It is advantageous to dissolve samples of dilute ointments (1:1000 or less) in xylene-acetic acid-alcohol solvent if the base is petrolatum or fat, and in plain water if the base is a jelly or gum. Add 2–3 Gm. of KBr, 0.2–0.3 Gm. $KBrO_3$ and 1–2 cc. of concentrated hydrochloric acid and digest the sample under a reflux on a steam bath for from fifteen to twenty minutes. To the cooled solution add pellets of KOH to an alkaline reaction, 0.2–0.3 Gm. zinc powder and 10–15 ml. of ethanolamine and reflux fifteen to twenty minutes longer. Filter off the zinc amalgam, dissolve in nitric, etc., as usual, cool, dilute, filter and make the clear nitric acid solution to a standard volume. Suitable aliquots can then be titrated by the dithizone procedure as indicated for dilute solutions and tinctures.

Often apparent shortages in dilute ointments packaged in metallic tubes are due to the reduction of mercury and subsequent amalgamation with the walls of the tube. Usually such amalgamation is easily determined by cutting open the tube and cleaning the walls of any residual ointment. A

TABLE III.—OINTMENTS

Sample No.	Preparation	Sample, Gm.	Mercury, Mg./Gm. Theory	Mercury, Mg./Gm. Label Claim	Mercury, Mg./Gm. Found	Recovery, %
1	Ammoniated Mercury	0.1720	....	7.0–9.0	7.45	...
2	Ammoniated Mercury	0.2370	....	3.5–4.5	3.80	...
3	Ammoniated Mercury[a]	0.2550	3.93	...	3.90	99.2
4	Ammoniated Mercury[a]	0.2100	7.86	...	7.85	99.8
5	Calomel[a]	0.6425	25.35	...	25.25	99.6
6	Calomel[a]	0.6215	25.35	...	25.35	100.0
7	Mercurial (Strong)	0.0880	....	47.5–52.5	48.2	...
8	Mercurial (Mild)	0.2500	....	9.0–11.0	10.2	...
9	Mercuric Oxide (Yellow)	0.5600	....	1.85–	1.88	...
10	Mercuric Oxide (Yellow)[a]	0.5150	0.925	...	0.920	99.5
11	Mercuric Oxide (Yellow)[a]	1.0205	0.925	...	0.922	99.7
12	Mercuric Nitrate	0.7710	....	7.0(?)	7.32	...
13	Mercuric Nitrate	0.5545	....	...	7.33	...
14	Mercuric Oleate	0.0781	....	24.0–26.0	25.3	...
15	Mercuric Oleate[a]	0.0580	25.8	...	25.7	99.6
			Mercury, γ/Gm.			
16	Mercuric Chloride[b,c]	3.40	....	148.0	90.0	...
17	Mercuric Chloride[a]	3.50	157.0	...	145.0	92.4
18	Mercuric Chloride[a]	3.50	157.0	...	150.0	95.5
19	Phenyl-Mercuric-Acetate	3.50	....	100.0	85.0	...
20	Phenyl-Mercuric-Acetate	4.40	....	100.0	88.0	...

[a] Samples prepared in laboratory from previously analyzed mercury compounds.
[b] Samples 16–20, inclusive, reduced by zinc-ethanolamine then titrated by dithizone procedure.
[c] Analysis of metal tube gave 370 γ mercury which, when added to total mercury in ointment in the tube, indicated 12.5% excess mercury in original ointment.

TABLE IV.—TABLETS AND POWDER MIXTURES

Sample No.	Preparation	Sample, Gm.	Mercurial, Mg./Tablet Theory	Mercurial, Mg./Tablet Label Claim	Mercurial, Mg./Tablet Found	Recovery, %
1	Calomel	1.000	...	32.0	31.0	...
2	Calomel[a]	1.250	31.85	...	31.8	99.8
3	Calomel and 6 Plant Extractives	2.500	...	129.6	126.5	...
4	Calomel and 6 Plant Extractives[a]	2.500	130.0	...	129.0	99.2
5	Calomel and 6 Plant Extractives[a]	1.850	40.4	...	40.3	99.5
6	Calomel and Soda	2.500	...	16.2	14.7	...
7	Calomel and Soda[a]	2.000	20.0	...	19.8	99.0
8	Mercury Bichloride	1.000	...	118.0	108.0	...
9	Mercury Bichloride[a]	1.000	120.0	...	119.4	99.5
10	Mercurous Iodide	2.000	...	16.2	15.3	...
11	Mercurous Iodide[a]	2.000	16.5	...	16.6	100.6

[a] Samples prepared in the laboratory from previously assayed mercurials with substances added to simulate the commercial products.

visual examination with the aid of a low-power lens will show clearly any amalgamated areas.

The amount of mercury amalgamated with the tube can be determined by cleaning the cut tube free of residual ointment, labeling, enamel, etc., and digesting with 1:1 nitric acid under an efficient reflux condenser, cool, wash down the reflux and filter off any metallic oxides. Wash the residue well with dilute nitric acid and precipitate the bulk of any lead with dilute sulfuric acid, again filter if necessary and wash the residue and filter well with dilute sulfuric. Add a few drops of 5% permanganate solution to give the filtrate a permanent purple color, reduce the excess permanganate with a few drops of dilute hydrogen peroxide and dilute to volume in a volumetric flask. Aliquots of the solution are then titrated by the dithizone procedure.

IV. *Tablets.*—The average weight of tablet preparations is obtained as usual by weighing about 20 tablets, then the sample is powdered to pass through a 60-mesh sieve. After thoroughly mixing the sieved powder, weigh portions calculated to yield 0.1 to 0.5 Gm. mercury into a small beaker, wet with a few drops of water and/or a few drops of HCl and grind to a smooth thin paste with a glass rod. Transfer the bulk of the paste to a 50- or 100-ml. volumetric flask with the aid of several small portions of glacial acetic. Treat the residual sample in the beaker with 5–10 ml. of glacial acetic and 5–10 drops of pure bromine. Transfer the bromine-acetic acid solution to the volumetric flask and rinse the beaker with several more small portions of acetic. Place the volumetric flask on the steam bath and heat carefully for three to five minutes so that the upper half of the neck does not become unduly warm to the fingers. If necessary, add more bromine to insure an excess of bromine in solution during the heating. If a small amount of insoluble residue persists after the heating and shaking of the flask add 25–30 ml. of glycerin, stopper the flask and thoroughly mix the solution. Cool to room temperature and dilute to volume with alcohol, stopper and again mix. Convenient aliquots (equal to 10–30 mg. mercury) of the well-mixed sample are then withdrawn, placed in the reduction flask and treated with pellets of KOH, ethanolamine solution, zinc dust, etc., as usual.

This method of obtaining representative samples has been found convenient for such preparations as Compound Cathartic Pills N. F. or the various commercial tablet mixtures of calomel and assorted vegetable extracts. The amount of tablet filler or diluents which does not actually dissolve by the above procedure is usually small and is easily kept in suspension while withdrawing the aliquots from the flask. Thus no appreciable error is introduced in the samples due to the unknown volume of the insoluble matter in the volumetric flask.

SUMMARY

The ethanolamine-reduction procedure of Rauscher (6) has been modified and applied to the analysis of a variety of mercury-containing preparations. The reducing action of the ethanolamine has been augmented by the addition of zinc which also serves to form an amalgam with the metallic mercury facilitating its quantitative recovery. The necessary precautions to obtain uniform representative samples for assay have been indicated.

Data have been presented which indicate that the revised procedure gives a rapid and accurate means of determining the mercury content of all mercurials ordinarily encountered.

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A Study of a New Salt of Neo-Synephrine*

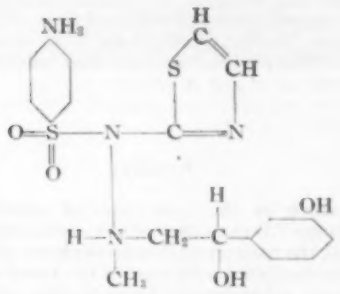
By C. W. Geiter, A. M. Lands and J. R. Lewis†

With the advent of sulfonamide compounds we investigated the possibility of combining one of them with the well-known vasoconstrictor, Neo-Synephrine Hydrochloride.[1] Realizing that the latter behaves as a base and the former as an acid, the senior author was successful in preparing a salt retaining the vasoconstrictor and bacteriostatic property of each component.

Neo-Synephrine (laevo-alpha-hydroxy-beta - methyl - amino - ethyl - 3 - hydroxy benzene)[2] was selected from the now large group of sympathomimetic compounds for its vasoconstrictor quality, its stability, duration of action and freedom from undesirable side reactions (1, 2). Sulfathiazole was chosen for the acid part of the molecule because of its clinical value (3, 4) and its relative freedom from side effects as compared with other similar members of the sulfonamide group. Under suitable conditions, these two substances unite to form an addition salt, Neo-Synephrine Sulfathiazolate, which separates from its solution in crystalline form (Fig. 1). The crystals are light orange in color, nonhygroscopic and odorless, with a melting point of 150° to 152° C. The solubility of this salt is shown in Table I.

EXPERIMENTAL

The structural formula of Neo-Synephrine Sulfathiazolate is:



Anal.—Calcd. for $C_{19}H_{22}O_4N_4S_2$: C, 51.5; H, 5.2; S, 15.2; N, 13.2. Found: C, 48.2; H, 4.9; S, 15.3; N, 13.2.

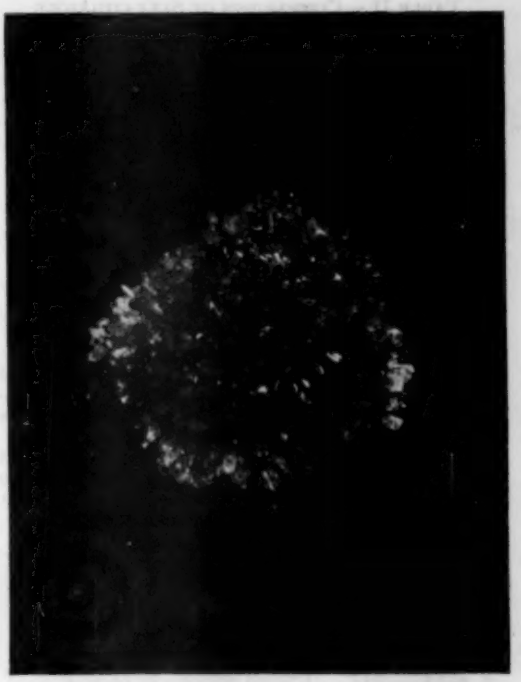


Fig. 1.—Neo-Synephrine Sulfathiazolate in crystalline form.

TABLE I.—SOLUBILITY OF NEO-SYNEPHRINE SULFATHIAZOLATE

Solvent	Gm./100 Cc.
Distilled water	2
Concentrated HCl	50
5 *N* HCl	10
Concentrated NaOH	Very soluble
0.01 *N* NaOH	20
Ethyl alcohol	10
Hot ethyl alcohol	20
Acetic acid	Insoluble
Hot acetic acid	5
Chloroform	Insoluble
Ether	Insoluble
Benzene	Insoluble

The Neo-Synephrine percentage and the vasoconstrictor potency of a 0.6% solution of Neo-Synephrine Sulfathiazolate are equivalent to those of the standard preparation of 0.25% Neo-Synephrine Hydrochloride.

A 0.6% solution of Neo-Synephrine Sulfathiazolate may be diluted with distilled water, 1% Neo-Synephrine Hydrochloride, physiological salt solutions, alkaline antiseptic solutions and almost any other type of solution that will not perceptibly re-

* Received April 12, 1944, from the Pharmacological Research Laboratory, Frederick Stearns and Company, Detroit, Mich.

† The authors wish to thank Dr. R. Feldkamp and Mr. W. Dobritt for their chemical assistance, and Mrs. E. Kerrigan for her bacteriological assistance.

[1] Registered U. S. Trademark.

[2] May also be designated as laevo(meta-hydroxyphenyl)-2 methylaminoethanol.

duce the *p*H, which is 7.4 in distilled water. Pontocaine hydrochloride, holocaine hydrochloride, zinc sulfate and benzocaine are not compatible, whereas cocaine hydrochloride may be added without difficulty. Solutions of iodine, neo-silvol and glycerin or propylene glycol are compatible.

An abbreviated comparison with sulfathiazole and its sodium salt is shown in Table II.

TABLE II.—COMPARISON OF SULFATHIAZOLE EQUIVALENTS

	Sulfathiazole	Sodium Sulfathiazole	Neo-Synephrine Sulfathiazolate
Molecular weight	255.21	278.207	422.308
% Sulfathiazole radical	100.00	91.78	60.41
% Solubility in water (at room temperature)	0.09	Over 30	2.00
Proportion of sulfathiazole	1.00	0.9	0.6

Comparative ultraviolet absorption spectra of aqueous solutions of Neo-Synephrine base, sulfathiazole and Neo-Synephrine Sulfathiazolate were made using a Beckman quartz spectrophotometer. The log ϵ (molecular extinction) values are plotted in Fig. 2. Both sulfathiazole and Neo-Synephrine Sulfathiazolate have wide absorption bands at approximately 258 and 278 millimicrons. However, the maximum extinction of Neo-Synephrine Sulfathiazolate is at 278 millimicrons, whereas the maximum extinction of sulfathiazole is at 258 millimicrons.

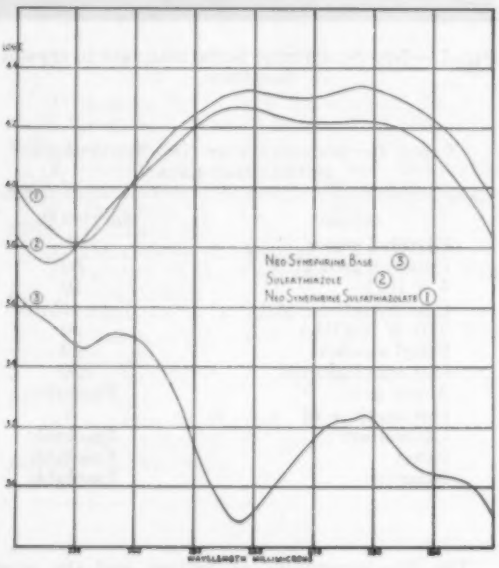


Fig. 2.—Ultraviolet absorption spectra of Neo-Synephrine base, sulfathiazole and Neo-Synephrine Sulfathiazolate in aqueous solution.

PHARMACOLOGY

Neo-Synephrine Sulfathiazolate contains 39.57% of Neo-Synephrine base, equivalent to 48% Neo-Synephrine Hydrochloride.

Using the direct blood pressure procedure on anesthetized dogs, Neo-Synephrine Sulfathiazolate injected intravenously produced a vasopressor response equal to that caused by a dose of Neo-Synephrine Hydrochloride, containing an equivalent amount of Neo-Synephrine base (Fig. 3). Repeated

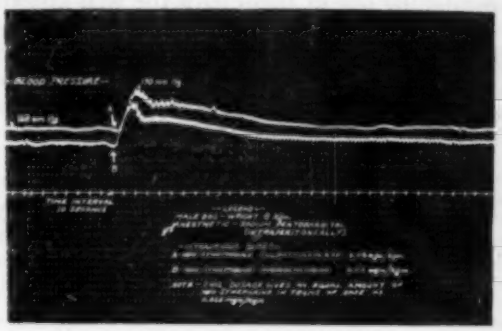


Fig. 3.

injections did not elicit any evidence of tachyphylaxis. Vasoconstrictor or blanching properties of Neo-Synephrine Sulfathiazolate are similar to those of corresponding amounts of Neo-Synephrine Hydrochloride when applied topically to the eyes of rabbits.

Studies of Neo-Synephrine Sulfathiazolate on ciliary activity were made using sections of ciliated membrane obtained from the frog (*Rana pipiens*). Comparative observations were made of the time required for a carbon particle to be moved over a distance of 0.5 cm. on ciliated pharyngeal sections bathed in either (*a*) Neo-Synephrine Sulfathiazolate 0.6% in 0.7% sodium chloride or (*b*) 0.7% sodium chloride alone. A series of carefully prepared sections did not show any significant differences in ciliary activity after being bathed in either of the two solutions. In addition, microscopic observations of small areas of ciliated cells were made, counting the number of pulsatile movements for thirty seconds after the cells had been bathed in the solutions. Sections bathed up to several hours in the Neo-Synephrine Sulfathiazolate solution did not give evidence of any ill effects.

TOXICITY

Inasmuch as the toxic dose of sulfathiazole is much larger (5) than that of Neo-Synephrine Hydrochloride, the toxicity of Neo-Synephrine Sulfathiazolate was compared with that of the latter substance. Both were administered by subcutaneous injection into albino rats of equal sex and weighing from 150 to 200 Gm. Thirty animals were used at each dosage level. The percentage of deaths was tabulated at the end of the fourth day. The doses of the Neo-Synephrine Sulfathiazolate were 60, 80 and 100 mg./Kg., whereas those of the Neo-Synephrine Hydrochloride (6) were 40, 45 and 50 mg./Kg. The LD_{50} of the Neo-Synephrine Sulfathiazolate was found to be 76 mg./Kg., and that of the hydrochloride to be 43 mg./Kg. (Table III). The results indicate that the new chemical union does not cause an increase in toxicity.

TABLE III.—TOXICITY OF NEO-SYNEPHRINE SULFATHIAZOLATE AND NEO-SYNEPHRINE HCl COMPARED

Drug	Dose, Mg./Kg.[a] 40	45	50	60	80	100
Neo-Synephrine Sulfathiazolate	...	...	...	13/30[b]	16/30	24/30
Neo-Synephrine Hydrochloride	14/30[b]	21/30	27/30	...	...	...

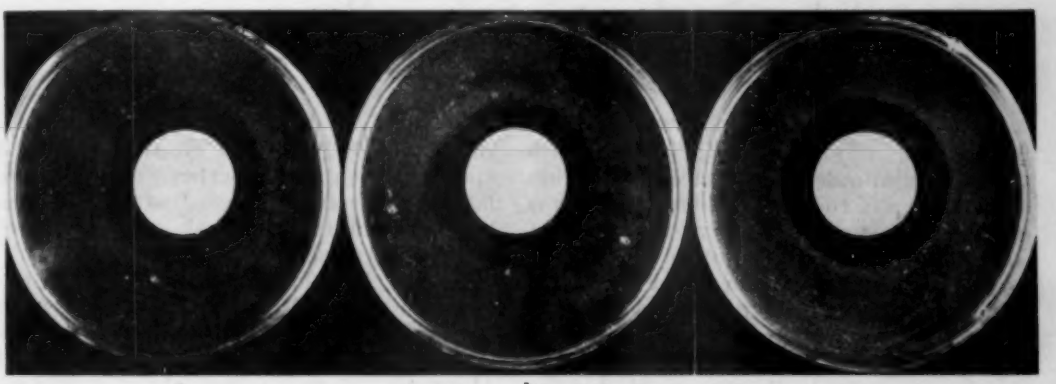
[a] All injections were made subcutaneously into albino rats. Survival was recorded for four days. The LD_{50} was obtained by plotting the Log Dose against % mortality.
[b] No. dead/no. injected.

BACTERIOLOGICAL STUDIES

Many of the methods used for studying the bacteriostatic action of the sulfa drugs are quite lengthy. Rake and McKee (7) reported on the agar-cup plate method (8) for testing sulfathiazole against *Staphylococcus aureus*. The method employed here is essentially the agar cup plate method with modifications as developed by Geiter. It is similar in basic procedure to the method introduced by Gershenfeld and Ibsen (9). This modified method has been used with good results in studying the activity of the sulfonamide drugs, giving a comparative evaluation in a relatively short period.

The procedure consists of inoculating the blood-agar medium with the test organism, pouring this into a Petri dish and allowing it to harden. A sterile circular piece of Whatman No. 1 filter paper, 2.5 cm. in diameter, is saturated (this size will absorb 0.2 cc. of aqueous fluid) with the solution to be tested and placed in the center of the plate with sterile forceps, care being taken to iron out any underlying air bubbles. The plates may be inverted in the incubator without loss of any of the solution being tested and with less tendency for the medium to dry and fissure than with the regular cup procedure.

Agar medium containing 20% to 25% of citrated blood or blood serum was used. Figure 4 shows the clear zones indicating the degree of bacteriostasis against a twenty-four hour standard culture of *S. aureus* (other organisms may be used) after twenty-four hours' incubation using 0.4% and 2.5% sodium sulfathiazole solutions and 0.6% Neo-Synephrine Sulfathiazolate solution. The 0.4% sodium sulfathiazole is equivalent in sulfathiazole content to a 0.6% Neo-Synephrine Sulfathiazolate solution. At the time of this writing the 2.5% solution of sodium sulfathiazole is the most popular percentage for topical use. During our many observations the area of bacteriostasis with the 2.5% solution was consistently similar to and only slightly greater than the area of bacteriostasis produced by a 0.4% sodium sulfathiazole solution. This suggests that possibly lower percentage strengths of the sulfathiazole salts may be adequate clinically. However, this remains to be proved inasmuch as microbiological laboratory tests cannot be relied upon to give the final answer.



a *b* *c*

Fig. 4. (*a*) 0.4% Sodium Sulfathiazole Solution. (*b*) 0.6% Neo-Synephrine Sulfathiazolate Solution. (*c*) 2.5% Sodium Sulfathiazole Solution.

CLINICAL STUDIES

By means of an atomizer with a small air compressor unit, a 1% solution of Neo-Synephrine Sulfathiazolate in a nebulizing vehicle was applied to the nose and throat of several hundred industrial workers (10). No signs or symptoms of irritation or sensitivity were produced. Most of these individuals were treated during the first stages (11) for a cold, two to three times a day for at least two days. Symptomatic relief was obtained usually within fifteen minutes after each application, with very few cases progressing to the secondary mucopurulent stage (12). In three cases of septic throat the symptoms became worse, and additional therapy was necessitated. Applications in some of the same individuals at a later date did not produce any untoward symptoms.

In those individuals unable to receive office treatment, a 0.6% Neo-Synephrine Sulfathiazolate aqueous solution was dispensed in a dropper bottle with instructions for use. A common observation was that a moist feeling in the throat and nose followed the use of these drops rather than the usual dry "crusty" sensation. There were no complaints of irritation or symptoms of sensitivity in these individuals.

Both of the above solutions had a *p*H of 7.8 to 8 and were approximately isotonic. The slight increase in *p*H is due to the preservatives added.

SUMMARY

1. A new salt of Neo-Synephrine, Neo-Synephrine Sulfathiazolate, is presented in this paper.

2. Pharmacological studies show that the vasoconstrictor properties of Neo-Synephrine is retained.

3. A solution of 0.6% Neo-Synephrine Sulfathiazolate with 0.7% sodium chloride did not affect ciliary activity.

4. A short method for determining comparative bacteriostatic powers of sulfa drugs is given.

5. Bacteriostatic qualities of sulfathiazole are retained in Neo-Synephrine Sulfathiazolate.

6. Limited clinical studies indicate that neither sensitivity nor irritation results from the repeated application of Neo-Synephrine Sulfathiazolate solution to the membranes of the nose and throat.

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Sclerosing Agents—Sodium Morrhuate*,†

By Howard Jensen‡ and Paul Jannke§

In 1815, Monteggio (1) advocated the injection of alcohol and lead acetate into varicosities in order to produce obliteration of the lumen of the vein. Since then, scores of sclerosing agents of varying degrees of suitability have been offered to the medical profession. Judging from the varieties of compounds which are known and used for this purpose, it can be concluded that no particular kind of substance and no specific structure is individually responsible for the sclerosing action. A survey of the literature reveals that a majority of physicians favor sodium morrhuate solutions. It is quite generally known, however, that the commercially available solutions of sodium morrhuate differ greatly in physical, chemical and therapeutic properties. Indeed, the late Dr. Bernard Fantus (2) stated that the lack of uniform chemical composition of sodium morrhuate is a great disadvantage.

It must be understood that sodium morrhuate is not a specific substance. Even though morrhuic acid ($C_9H_{13}NO_3$) was clearly described in 1888 by Gautier and Mourgues (3) as a monocarboxylic acid related to pyridine, the modern conception of sodium morrhuate is in a state of confusion. It is loosely defined as being: "the sodium salt of the fatty acids extracted from cod liver oil" (4, 5, 6); "the sodium salt of the unsaturated fatty acids from cod liver oil" (7, 8); "the sodium salt of purified cod liver oil" (9); "the sodium salt of fractionated cod liver oil" (10).

The term was apparently coined by Rogers (11) who introduced the complex substance in the treatment of tuberculosis, wherein he injected intravenously a three per cent solution of the sodium salt of the fatty acids of cod-liver oil. In the following year, 1919, the compound with which Rogers worked (12) was identified as the sodium salt of the unsaturated fatty acids only. Higgins and

* Received January 15, 1944, from the College of Pharmacy, University of Nebraska, Lincoln, Neb.

† The authors wish to express their appreciation for samples and other cooperation given them in this project by George A. Breon and Co., Burroughs Wellcome and Co., Crookes Laboratories, Inc., The Drug Products Co., Endo Products, Inc., Harvey and Co., Kremers-Urban Co., The Lakeside Laboratories, Eli Lilly and Company, The Loeser Laboratory, Wm. S. Merrell and Co., Miller and Co., Parke, Davis and Co., G. D. Searle and Co., Sherman and Co., Ulmer and Co. and the Upjohn Company.

‡ AMERICAN PHARMACEUTICAL ASSOCIATION Fellow, 1942.

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Kittel (13), of England, reported their use of sodium morrhuate as a sclerosing agent. Even in its ill-defined form, sodium morrhuate was formally recognized (14) by the

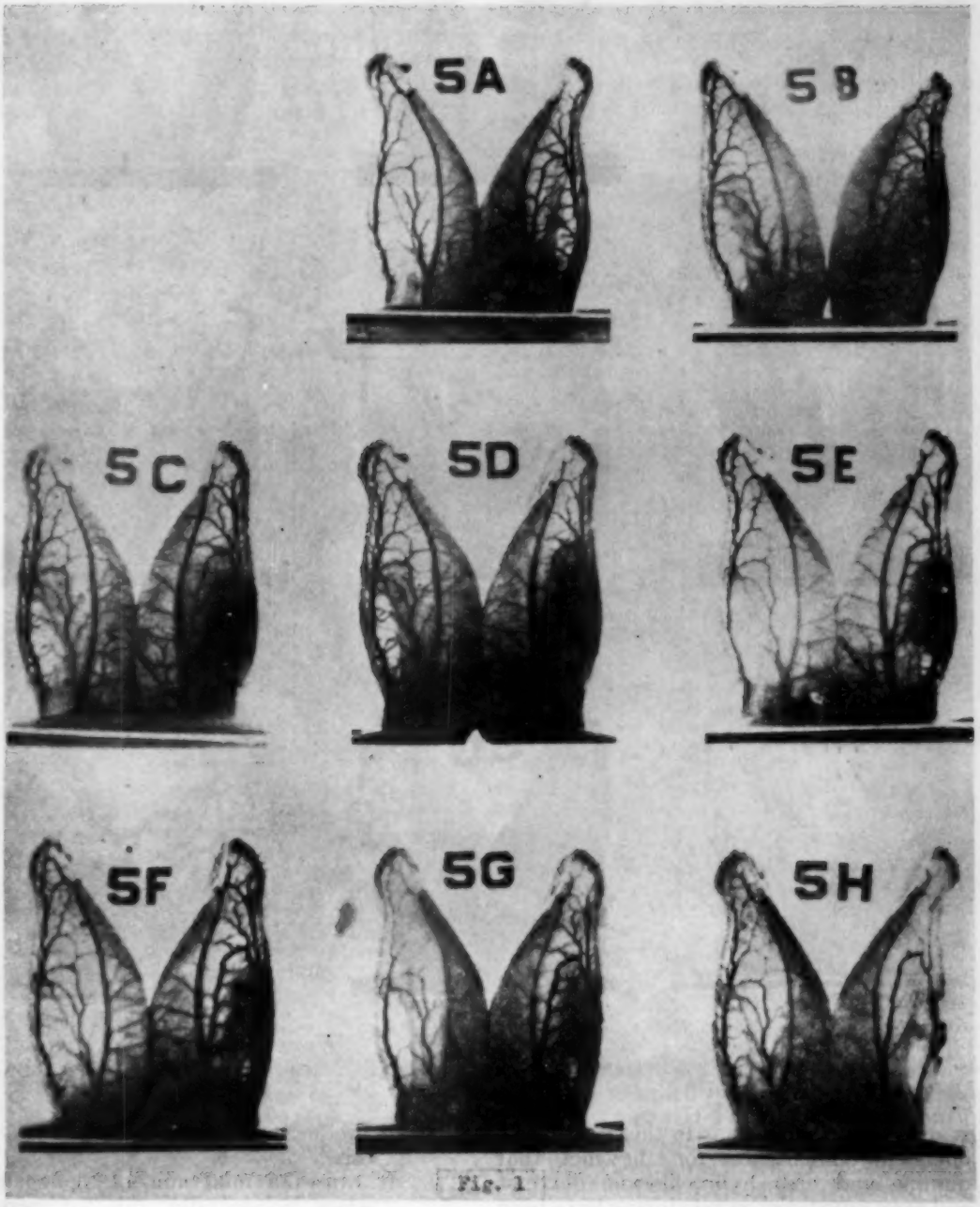


Fig. 1

medical profession in the United States, in 1934, as a sclerosing agent. Tests for purity and identity were assigned to it.

Among the acids which are known to be in cod-liver oil are: acetic, butyric, valeric, capric, myristic, palmitic, stearic, oleic, linoleic, linolenic, erucic, clupandonic, aselic, gadinic, jecoric, jecoleic, therapic, zoomaric and morrhuic. To date, no one has ventured to attribute sclerosing action to the sodium salt of any one acid. In one reference (15), it is indicated that this action

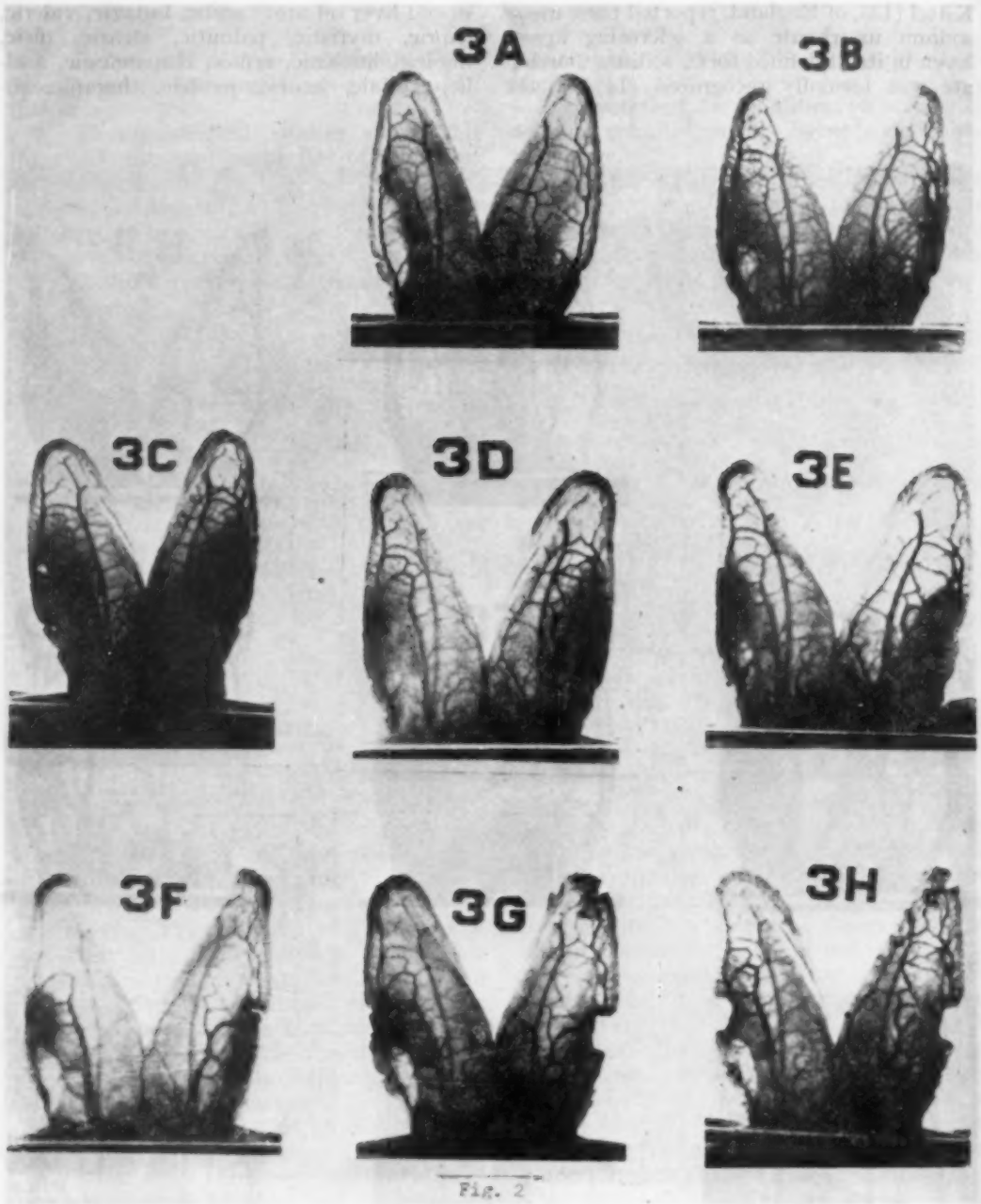


Fig. 2

is due to sodium ions. If this be true, it offers no explanation for the fact that quinine and urea hydrochloride, dextrose and even mucilaginous substances have been used effectively in the treatment of varicose veins.

Though the solution of sodium morrhuate has been widely used in the obliteration of varicosities, it is not free from adversities. Those who favor it offer these features:

1. It produces positive, permanent sclerosis.
2. It causes a minimum of sloughing when accidentally injected perivascularly.
3. It causes a minimum of pain and local irritation.
4. Action is very prompt and easily localized.
5. More injections made at one sitting.

6. Patients need not take a reclined position during the time of injection nor for a prolonged period thereafter.
7. Solutions are stable.
8. It has no contraindication.

The most common objection to sodium morrhuate is the fact that the solutions are not standardized; their properties and their identities vary greatly among the different pharmaceutical manufacturers. Less common, nevertheless worthy of the greatest consideration, are the reports of anaphylactic shock (16) following the injection of this sclerosing agent.

Quite naturally, the pharmaceutical manufacturers have given adequate consideration to the undesirable features of the medicament. Attempts to eliminate them have been met with varying degrees of success. It is possible that the anaphylactic shock is comparable with a foreign protein shock, since the presence of nitrogen-containing acids in cod-liver oil has been demonstrated. Experiments are now being conducted in an attempt to remove the amino acids before the cod-liver oil is saponified, Attempts are also being made to isolate and to identify the substances which cause anaphylactic shock. Qualitative tests for amino acids in commercial sodium morrhuate are still in the experimental stage. From the congealing points of the solutions (see Table I) it is is obvious that the majority of pharmaceutical manufacturers have effected a so-called fractionation of the acids isolated from cod-liver oil with the aim of removing the saturated acids. The need for this operation is based on the supposition that the sodium salts of the saturated acids are more irritating than the salts of the unsaturated acids, and that their sclerosing action is comparatively low. Experimental data compiled in this laboratory on various fractions of the acids are contradictory to the supposition. It was found that although the salts of the less-saturated acids

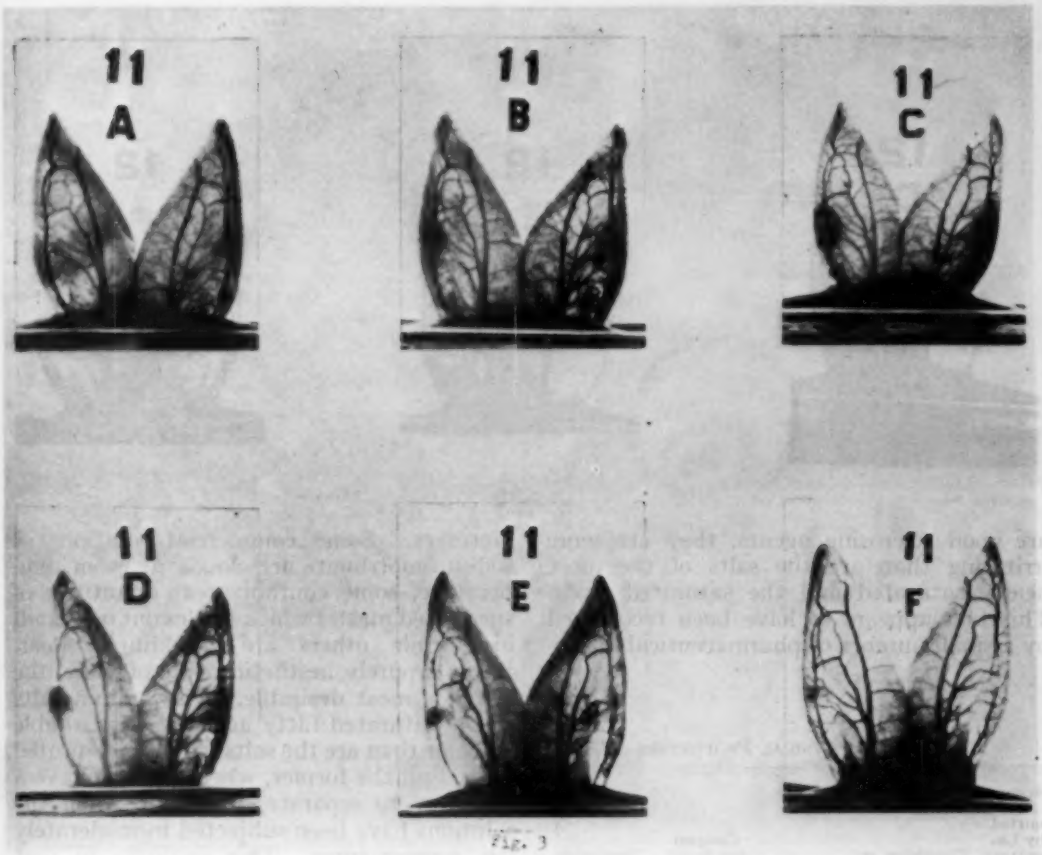


Fig. 3

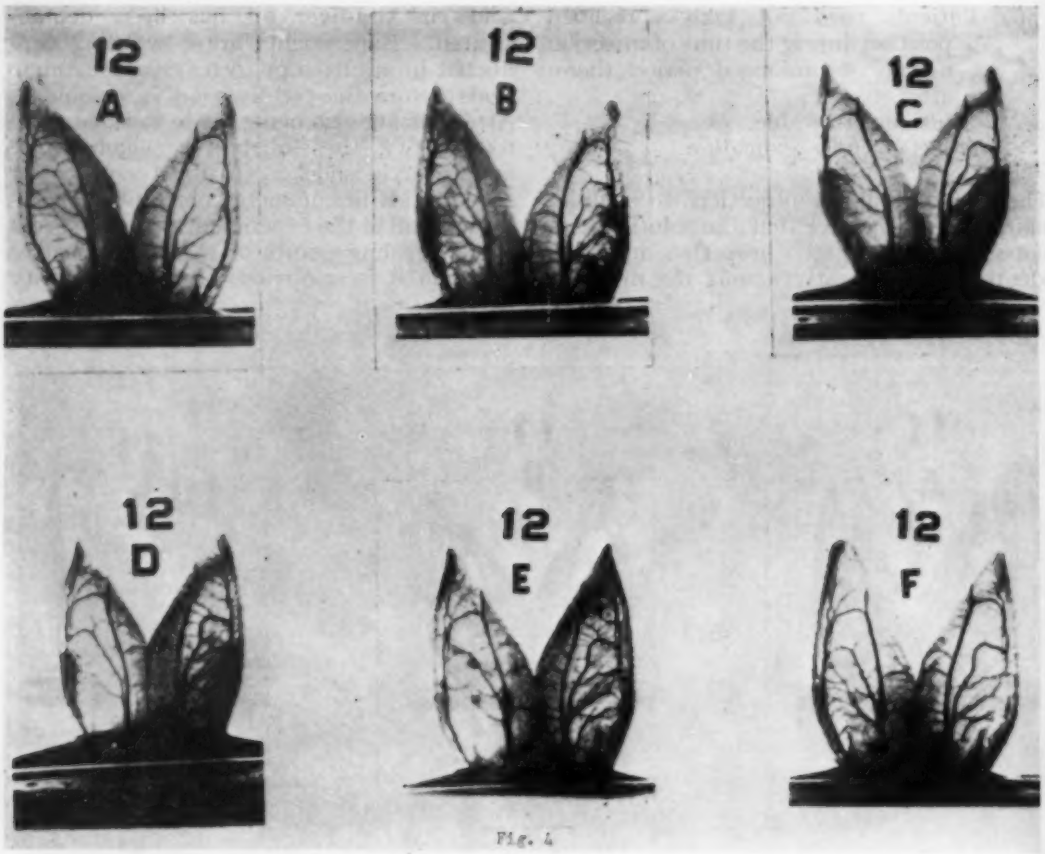


Fig. 4

are good sclerosing agents, they are more irritating than are the salts of the more nearly saturated and the saturated acids. This fact appears to have been recognized by a small number of pharmaceutical manufacturers. Some commercial solutions of sodium morrhuate are cloudy at room temperature, some contain great quantities of suspended matter which settles out on standing, while others are sparklingly clear. From a purely aesthetic point of view, the latter is most desirable. The sodium salts of the saturated fatty acids are less soluble in water than are the salts of the unsaturated acids, and the former, when present, have a tendency to separate, especially when the solutions have been subjected to moderately low temperatures. This accounts in part for the cloudiness, but it must be remembered that unless the unsaponifiable fraction of the oil has been completely removed, it will contribute to the heterogeneity of the solution. Two of the commercial samples examined in the experimental work contained, as of the label, two and three per cent, respectively, of alcohol which was obviously incorporated to insure the clarity of the solutions at all reasonable temperatures.

TABLE I.—PHYSICAL PROPERTIES

Sample Submitted by Laboratory No.	Condition at Room Temp.	Color	Congealing Point, °C.	Free acids at 0° C.
1	Precipitate	Dark	7	Liquid
2	Clear	Light	−8	Semisolid
3	Clear	Medium	<−11	Liquid
4	Clear	Dark	−8	Liquid
5	Clear	Very light	−3	Solid
6	Clear	Medium	<−11	Liquid
7	Clear	Medium	−8	Solid
8	Cloudy	Light	−3	Solid
9	Clear	Medium	−3	Solid
10	Clear	Light	−8	Solid
11	Clear	Light	<−11	Solid
12	Cloudy	Dark	3.5	Semisolid
13	Cloudy	Medium	0.5	Solid
14	Cloudy	Dark	7	Semisolid
15	Precipitate	Dark	7	Solid
16	Clear	Medium	−6	Solid
17	Cloudy	Light	3.5	Solid

EXPERIMENTAL

In addition to solutions of sodium morrhuate

prepared in the laboratory, samples of 5% strength submitted by 17 manufacturers were examined physically, chemically and pharmacologically. Two of the commercial samples contained dense precipitates at room temperature, 5 were cloudy and the remainder were clear. The colors described in Table I range from light (yellow) through medium (yellow) to dark (light brown). To determine the congealing points recorded in Table I, the samples were exposed to the range of temperatures indicated, during an interval of twenty-four hours each. The condition of the free fatty acids at 0° C. is also described. The source of these acids will be described under the assay procedure.

brated portion of the neck, and the sample was centrifuged for twenty minutes at a speed of 1500 r. p. m. After cooling to room temperature, the volume of the free fatty acids was measured. The physical state of the free acids at 0° C. is indicated in Table I. Calculations are based on the specific gravity of morrhuic acid ranging from 0.898 to 0.907, averaging 0.9025. It is reported that sodium morrhuate consists of from 7.0 to 7.8% of sodium and from 92.2 to 93% of morrhuic acid (17). The percentage of the labeled amount of sodium morrhuate was calculated on the basis of 92.6% of morrhuic acid.

A potential source of error in this method of assaying is based on the fact that the oily acids have a

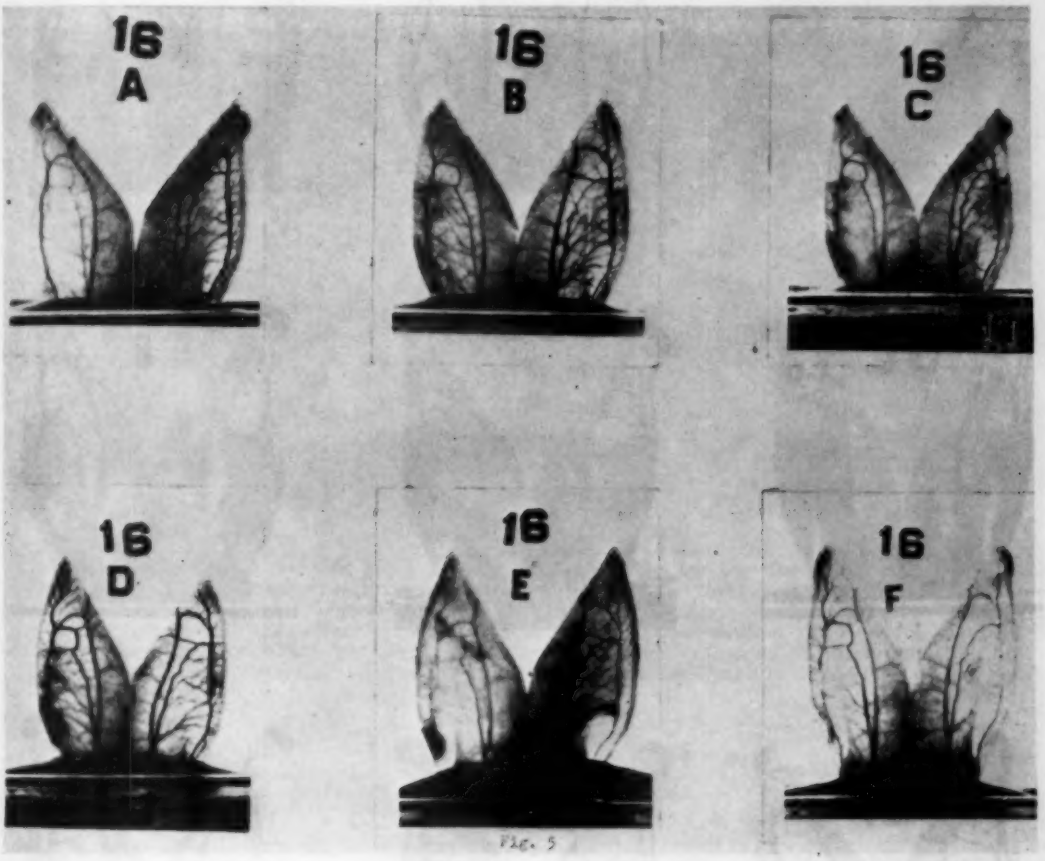


Fig. 5

The chemical study of the solutions was limited to the determination of the *p*H values, the assay for the sodium morrhuate content, the titration of the free fatty acids to determine the sodium content of the sodium salts, and the determination of the iodine value of the free acids.

In order to ascertain the amount of sodium morrhuate,[1] an accurately measured 5-cc. sample, representing 0.25 Gm. of salt, was placed in a Babcock bottle and acidified with 2 cc. of 10% sulfuric acid solution. Sufficient hot water was added to bring the volume of the free fatty acids within the cali-

tendency to cling to the tapering shoulder of the Babcock bottle. Furthermore, the oil column in the neck is small, and an error in the estimated volume is greatly magnified in calculating the result. A modification of this method is being studied using ligroin as a diluent for the free acids and will be reported at a later date.

The sodium content of the regenerated salts was calculated from the titration of the free acids with standard alcoholic sodium hydroxide solution. The free fatty acids which separated from acidified samples of the sodium morrhuate solutions were removed with a small pipette, and dried over anhydrous sodium sulfate. Accurately weighed samples of the free acids were dissolved in neutral alcohol and titrated with half-normal alcoholic sodium hydroxide solution in the presence of phenolphthalein T

[1] Slight modification of a method described by Dr. C. W. Sondern, George Breon Co., Kansas City, Mo., in a personal communication.

S. The percentage of sodium in the sodium morrhuate thus formed was calculated. The titration mixtures were diluted with sufficient water to make a 5% solution of sodium morrhuate. The *p*H of these solutions was determined to provide a comparison with the *p*H of the original solutions. The differences between these values may, in part, be attributed to the fact that the solutions showed a strong affinity for carbon dioxide. See Table II.

Haines (18) reports the iodine value of pure morrhuic acid as 226.5. Of the 17 commercial samples assayed, only 2 were in agreement with this value. The N. N. R. indicates that the iodine value lies between 145 and 185 (19). Eleven commercial samples have iodine values between these extremes.

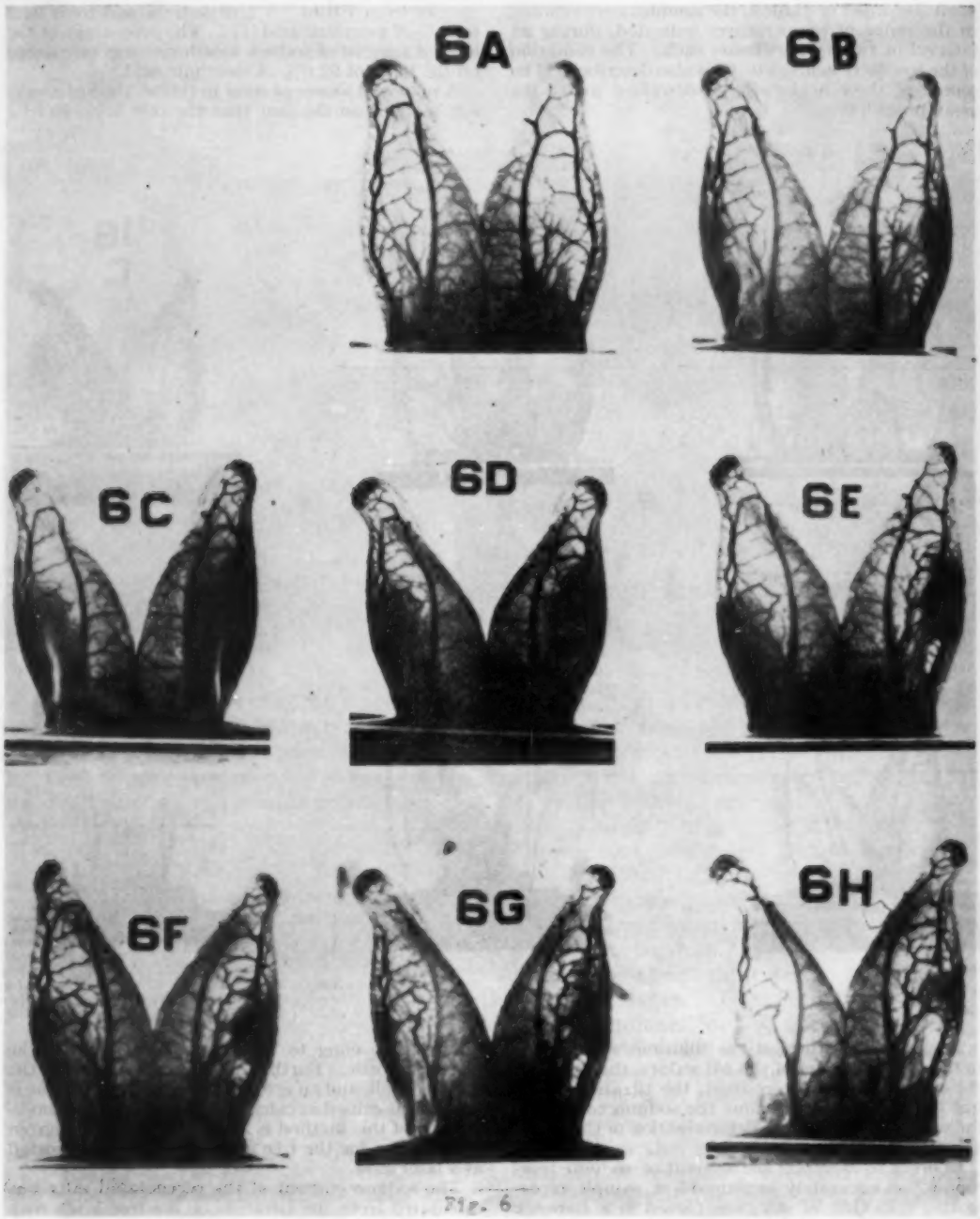
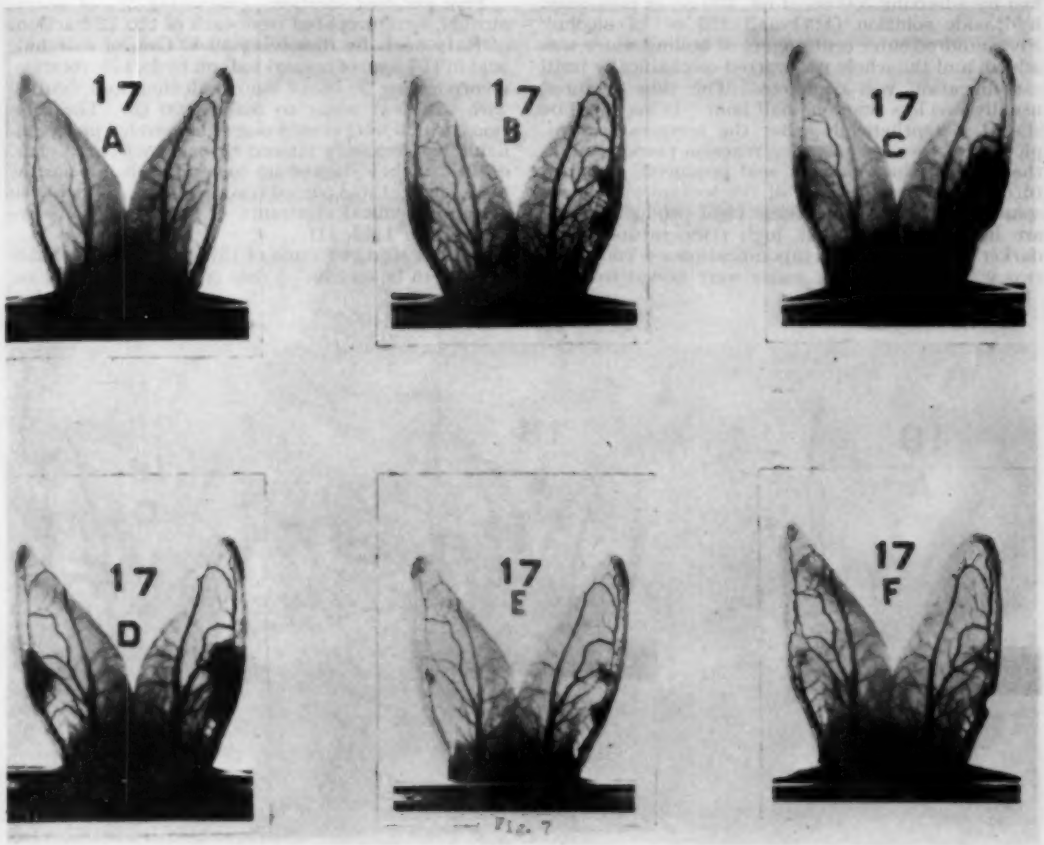


Fig. 6

The iodine value of the free fatty acids, dried over anhydrous sodium sulfate, was determined by the micro-method described by Ralls (20).

Solutions of sodium morrhuate prepared in the laboratory were made from the acids isolated from



— Fig. 7

plain and from modified cod-liver oils. Modifications were rendered in two ways, namely, by extraction with aqueous 1% hydrochloric acid, and by extraction with alcoholic 1% hydrochloric acid. These extractions were made in an attempt to remove from the oil the nitrogen-containing substances which presumably are the cause of anaphylactic shock. The extracts themselves were reserved for later study. The specific substance known as morrhuic acid was isolated from the alcoholic hydrochloric acid extract by Gautier and Mourgues (21).

Each of the 3 samples of cod-liver oil was saponi-

TABLE II.—CHEMICAL PROPERTIES

Sample Submitted by Laboratory No.	ρH of Samples	Morrhuic Acid, Cc.	Sodium Salt, Gm.	Labeled Amount of Sodium Morrhuate, %	Iodine No.	Sodium by Titration, %	ρH of Titrated Solutions	Sodium Salt by Titration, Gm.	Sodium Morrhuate by Titration, %
1	9.35	0.250	0.24365	97.46	176.46	6.85	8.64	0.24221	96.88
2	10.21	0.236	0.23001	92.04	165.71	6.96	9.85	0.22892	91.56
3	9.80	0.226	0.22026	88.10	227.28	7.03	9.46	0.21939	87.75
4	9.60	0.226	0.22026	88.10	141.83	6.95	9.24	0.21920	87.68
5	10.65	0.226	0.22026	88.10	156.39	6.95	10.69	0.21920	87.68
6	9.43	0.210	0.20467	81.87	227.35	7.01	9.35	0.20381	81.52
7	9.60	0.236	0.23001	92.04	181.60	6.95	10.00	0.22889	91.55
8	10.00	0.210	0.20467	81.87	146.05	7.05	9.41	0.20389	81.55
9	10.13	0.240	0.23391	93.56	152.75	6.92	9.74	0.23270	93.08
10	10.10	0.240	0.23391	93.56	152.97	6.95	9.72	0.23277	93.10
11	10.50	0.230	0.22416	89.66	152.78	7.02	10.01	0.22324	89.29
12	9.21	0.200	0.19492	77.97	126.83	7.05	8.91	0.19419	77.67
13	9.70	0.232	0.22611	90.44	123.60	7.25	9.49	0.22574	90.29
14	9.53	0.226	0.22026	88.10	158.20	7.23	9.13	0.21986	87.94
15	9.24	0.216	0.21052	84.20	122.31	7.16	8.91	0.20997	83.99
16	9.80	0.226	0.22026	88.10	154.39	7.23	9.33	0.21986	87.94
17	9.51	0.236	0.23001	92.04	148.54	7.14	9.20	0.22913	91.65

fied by admixing 500 cc. of oil, 250 cc. of potassium hydroxide solution (1:1) and 125 cc. of alcohol. Five hundred cubic centimeters of boiling water was added, and the whole was stirred mechanically until saponification was complete. The time required usually was less than one-half hour. It may well be stated, parenthetically, that the temperature employed for the saponification reaction predetermines the color of the morrhuic acid produced, which in turn determines the color of the sodium morrhuate solutions. Low temperatures yield products which are light in color, while high temperatures yield darker products. When saponification was complete, two volumes of boiling water were added to completely dissolve the soaps, and the cloudy, yellow solution was rendered faintly acid with 10% hydrochloric acid. The aqueous layer which separated was siphoned off, and the free fatty acids were washed with 2 portions of boiling, saturated salt water, and finally with distilled water to remove chloride.

Each of the mixtures of fatty acids isolated from the 3 kinds of cod-liver oil, plain, aqueous acid extracted, and alcoholic acid extracted, was dried over anhydrous sodium sulfate and then fractionated by exposure to consecutively lower temperatures. Fractions were collected at 10° C., −10° C., and −15° C. Thus, each mixture of fatty acids was resolved into 4 parts, namely, those acids which were solid at each of the 3 temperatures indicated and those which remained liquid at −15° C.

Five per cent solutions, containing 2% of benzyl alcohol, were prepared from each of the 12 fractions of fatty acids by dissolving 46.40 Gm. of morrhuic acid in 156.5 cc. of normal sodium hydroxide solution, incorporating 20 cc. of benzyl alcohol, and diluting with sufficient water to make 1000 cc. The solutions, which were cloudy due to suspended unsaponifiable matter, were filtered through activated charcoal until they showed no turbidity when a narrow beam of light was passed through them. The physical and chemical constants of these solutions are recorded in Table III.

From a study of some of the data listed in Table III, it can be concluded that treatment of cod-liver oil with alcoholic hydrochloric acid and with aqueous hydrochloric acid produced little, if any, change in the chemical constitution of the oil. This is most clearly indicated by the uniformity of the iodine values and the percentage of sodium in the sodium salts of the acids. The comparison is drawn in Table IV.

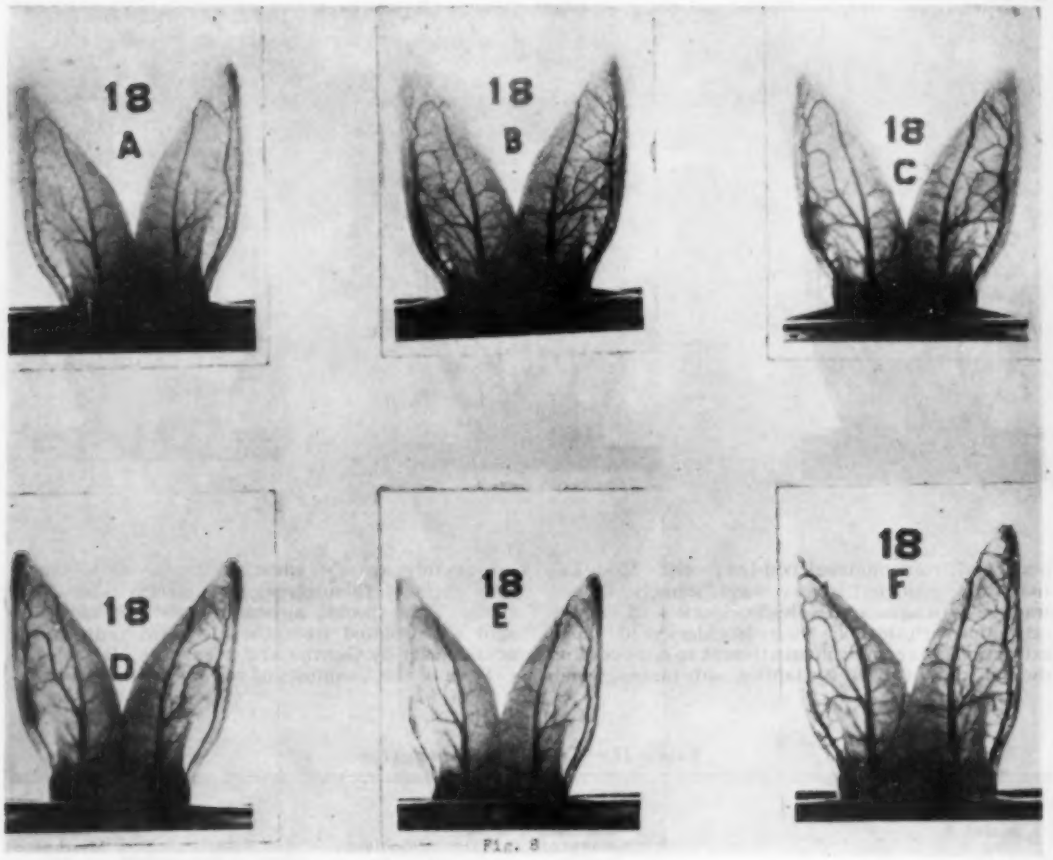


Fig. 8

The method described by Isaak (22) for the pharmacological studies of sclerosing agents was found to be ideal after a few changes in technique were applied. The method is based on the injection of solutions of sclerosing agents into the marginal vein of the rabbit's ear and recording the progress of sclerosis photographically. Albino rabbits weighing between 3 and 6 pounds were selected for this purpose because their ears are not as dense as are those of larger animals, and the hair on the ears is not long

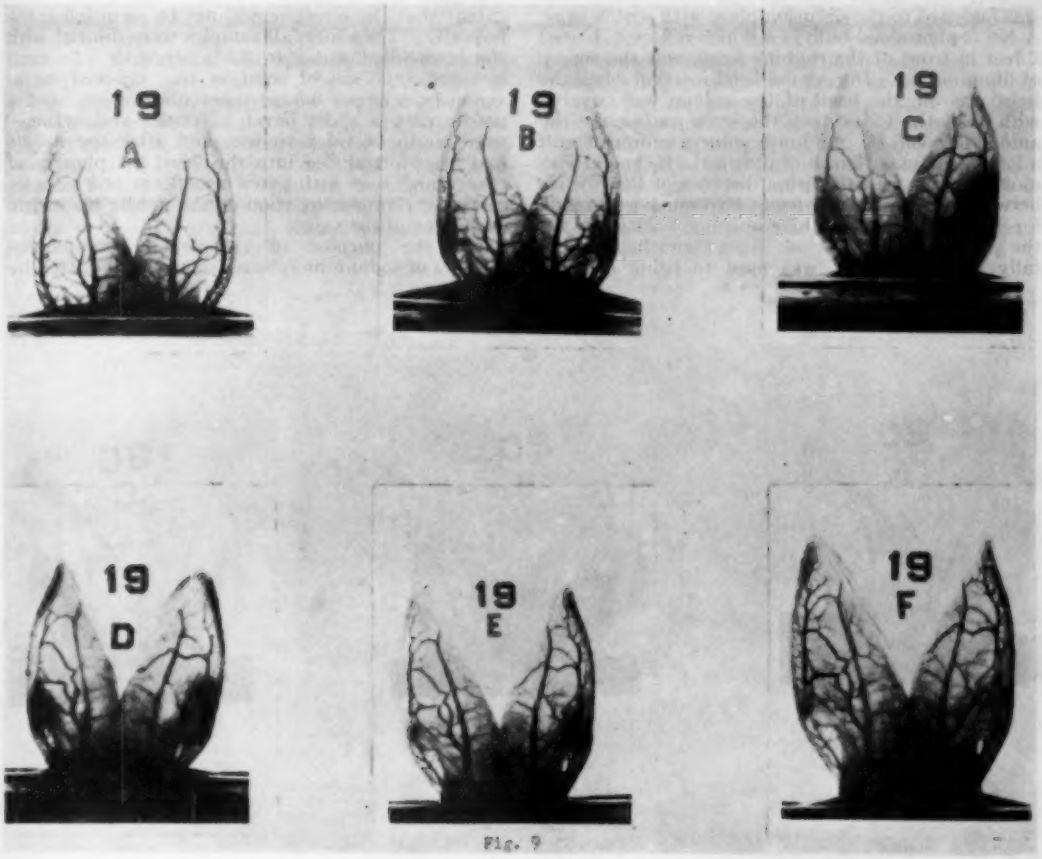


Fig. 9

TABLE III

Sample[a]	Acid Fraction	Congealing Point of Solution of Sodium Salt, ° C.	pH of 5% Solution of Sodium Salt	Sodium Morrhuate, %	Sodium, %	Sodium Morrhuate by Titration, %	Iodine Value
− 1	Liquid, −15° C.	<−16	9.89	103.36	6.90	101.60	184
2	Liquid, −15° C.	<−16	9.62	101.39	6.98	99.74	179
3	Liquid, −15° C.	<−16	9.88	100.60	6.98	98.96	178
− 4	Solid, −15° C.	<−16	10.27	102.58	7.00	100.92	163
5	Solid, −15° C.	<−16	10.64	101.76	6.94	100.08	165
6	Solid, −15° C.	<−16	10.20	104.55	7.03	102.89	169
− 7	Solid, −10° C.	3.5	10.16	102.58	7.06	100.98	127
8	Solid, −10° C.	3.5	10.18	101.76	7.19	100.35	132
9	Solid, −10° C.	3.5	10.14	97.45	7.18	96.06	128
−10	Solid, +10° C.	7.0	10.53	100.61	7.23	99.23	119
11	Solid, +10° C.	7.0	10.35	96.66	7.27	95.38	111
12	Solid, +10° C.	7.0	10.26	103.36	7.32	102.05	123

[a] Samples 1, 4, 7 and 10 were prepared from plain cod-liver oil.
Samples 2, 5, 8 and 11 were prepared from alcoholic-acid extracted cod-liver oil.
Samples 3, 6, 9 and 12 were prepared from aqueous-acid extracted cod-liver oil.

enough to necessitate clipping. It was found that painting the ears lightly with mineral oil greatly facilitated the transmission of light through the nonvenous tissues. The oil was applied with a swab, immediately prior to the taking of the pictures, and the ears were dried with a soft cloth promptly thereafter. For photographing and injecting, the rabbit was placed in a rabbit box, with the ears projecting in the usual manner. The ears were held together, erect and stationary by fastening together the two inner edges with a small paper clip placed about an inch above the base of the ears. An opal glass diffusion panel was mounted on the box, directly in front of the ears. The serial number of each rabbit

was fastened to the diffusion plate with scotch tape. A No. 2 photoflood bulb in a 6-inch reflector, placed 2 feet in front of the rabbit's head, was the source of illumination. During the brief interval when the light was on, the head of the animal was covered with a cloth, to protect the eyes and spare the animal discomfort. A 35-mm. camera equipped with a 2.8 Zeiss Tessar lens and portrait attachment was mounted on a tripod, with a distance of 13.5 inches between the ears and the lens. Exposures were made for 1 second, with aperture opening *f*-22 to obtain the greatest depth of focus. Agfa Plenachrome film, fully orthochromatic, was used to bring out the maximum contrast between the red blood vessels and the lightly colored cartilage. Since the film is highly sensitive to red, blood vessels and irritated areas appear solidly black in the prints. Obliterated blood vessels are yellowish, and therefore they are not visible in the pictures.

Normal printing conditions were altered in order to emphasize contrast in the finished pictures. Film strips were developed for twenty-five minutes at 60° F., in Defender 777 developer. Three-inch by 5-inch projection prints were made on Defender R4 single weight, glossy paper, a paper with high contrast qualities. They were developed in Defender 53 paper developer which was diluted 2:3, instead of the usual 2:1, to add to the contrast in the pictures.

The 5% solutions, even when very small volumes were injected, proved to be overly active to such an extent that the results could not be recorded satisfactorily. Therefore, all samples were diluted with sterile distilled water to 2.5% strength. In each instance, 0.05 cc. of solution was injected intravenously, using a 0.5-cc. tuberculin syringe and a needle with a short bevel. Needles and syringes were sterilized prior to use, and after the needle had been introduced into the vein, the plunger of the syringe was withdrawn a trifle in order to ascertain the proper location of the needle tip within the lumen of the vein.

For the purpose of comparison, commercial samples of sodium morrhuate solution submitted by

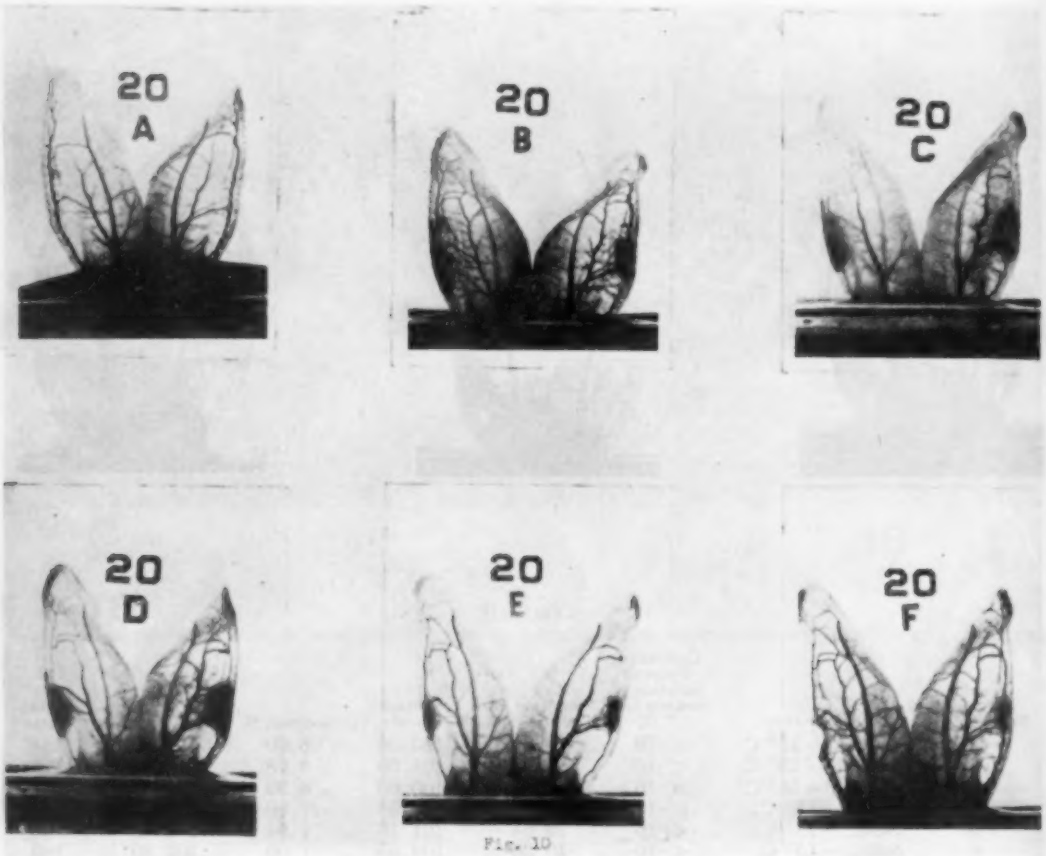


FIG. 10

TABLE IV

Sample	Iodine Value	Sodium, %
1	184	6.90
4	163	7.00
7	127	7.06
10	119	7.23
2	179	6.98
5	165	6.94
8	132	7.19
11	111	7.27
3	178	6.98
6	169	7.03
9	128	7.18
12	123	7.32

Laboratory No. 5 were selected as the standard. These samples were selected as the standard solely because of the fact that they appeared to be average, in so far as physical and chemical properties are concerned. In each instance, the standard was injected into the left ear, and the sample to be compared was injected into the right ear. Prior to making the injection, a wire test tube clamp, which had been bent flat on the gripping ends and whose spring tension had been greatly reduced, was attached to the ear in such a manner as to block the circulation in the area where the medicament was to be introduced. The solution was injected slowly to avoid unnecessary pressure in the vein, and after two minutes, the clamp was removed.

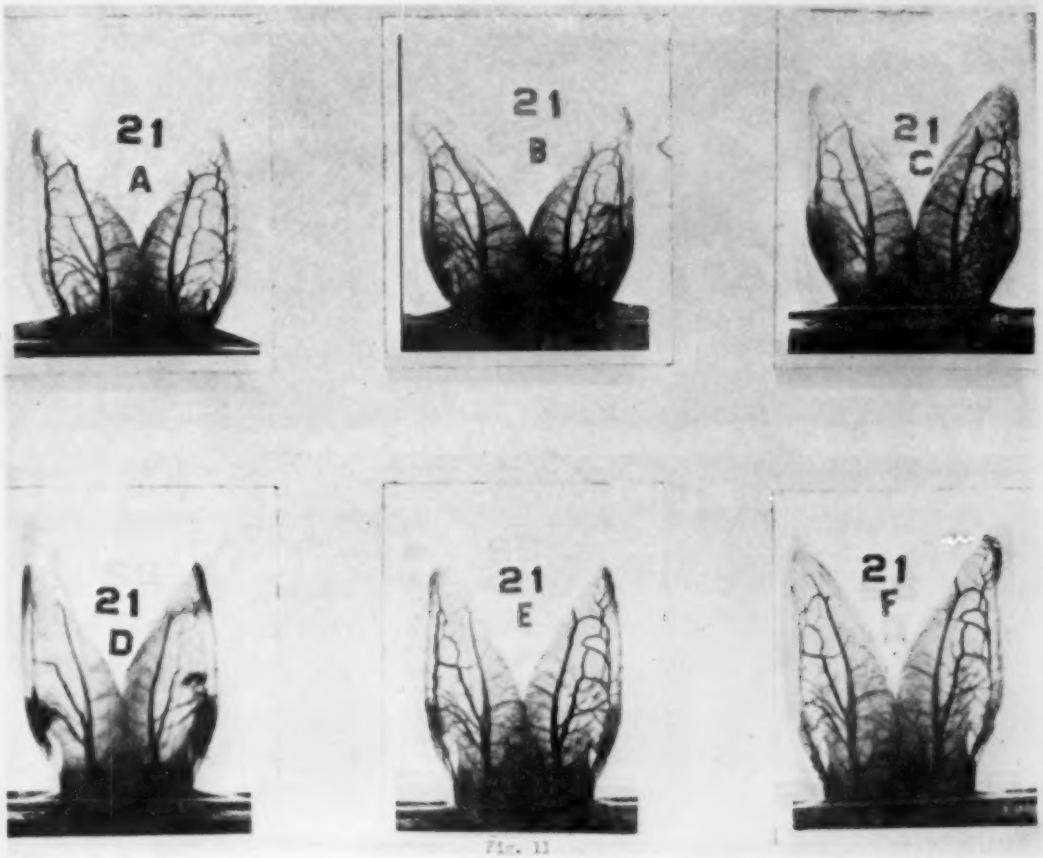


FIG. 11

All ears were photographed in their normal state (picture *A* in all series). At first, pictures were taken after injection, at intervals of fifteen minutes, one hour, two hours, twenty-four hours, three days, two weeks and four weeks in order to follow the progress of the action. When it was observed that the change occurring at the one-hour and two-hour intervals was not significant, exposures at these two intervals were abandoned. This accounts for the fact that in a few series, there are eight photographs, while in other series there are six.

Variations in pharmacological properties of the commercial samples were found to be as marked as were the physical and chemical variations. Bearing in mind the supposition that the effectiveness of the sclerosing agent is directly proportional to the degree of unsaturation of the fatty acid, one would expect to find a maximum of sclerosis and a minimum of irritation resulting from the injection of samples containing acids with an iodine number of 227. Figures 1 and 2 show the results of these injections. In series 5, the blank (left ear) with an iodine value of 156 shows a slight, but definite sclerosis, with an unusually small amount of irritation, while the sample being compared (right ear) shows a great amount of irritation with a slight, inconspicuous sclerosis. Series 3 shows a greater degree of irritation in the case of the test sample as compared with the standard. The reason for the sections of the ears breaking out is not clearly understood, but it is not attributed to an over-activity of the sclerosing agent. It may be the result of a traumatic condition, or the rabbit may have scratched its ears when they were in a highly irritated state.

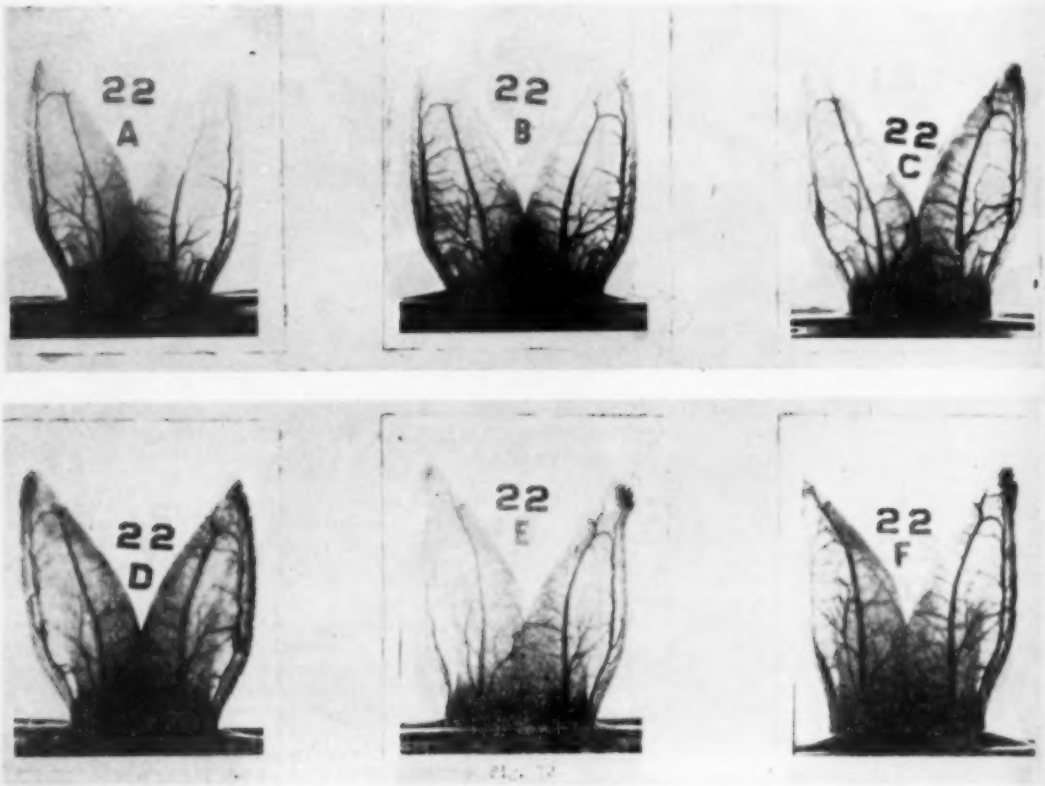
Figures 3 and 4 illustrate the results following the injection of solutions of sodium morrhuate wherein the fatty acids had low iodine values, namely, 126 for series 11, and 123 for series 12. It is here that one would expect to find the greatest amount of irritation and the least of sclerotic action, since the proportion of saturated fatty acids in the sodium

morrhuate is high and the proportion of unsaturated acids is low. Instead, the results show smaller effected areas, lower degree of irritation and a positive sclerosis in both cases.

It was previously indicated that according to the N. N. R., the iodine value for the free fatty acids should lie between 145 and 185. Figures 5 and 6 are illustrative of these two extremes. Series 16 corresponds to iodine value 148, and series 6 corresponds to iodine value 181. In the case of the former, the effected area is very small, and the irritation slight, but the sclerosis is positive. In the case of the latter, the extent and degree of irritation are greater and the sclerosis is less obvious, except for the development of communicating vessels. It should be pointed out that in all illustrations of the higher iodine values, the site of irritation had not definitely returned to its normal condition at the time when the last photographs were taken. This would indicate that the greater degree of unsaturation produces a prolonged irritation in addition to a more intensified irritation.

Figures 7 through 18 show the sclerosing action of solutions of sodium morrhuate prepared in the laboratory. They clearly demonstrate two points: treatment of cod-liver oil with alcoholic or aqueous hydrochloric acid offers no advantage in so far as sclerosing action is concerned; sodium morrhuate prepared from the more highly saturated fatty acids has definite sclerosing properties which are accompanied by a lesser degree of irritation than is incurred in conjunction with sodium morrhuate prepared from the more unsaturated fatty acids. Series 17, 20, 23 and 26 correspond to samples 1, 4, 7 and 10 (Table III) prepared from plain cod-liver oil; series 18, 21, 24 and 27 correspond to samples 2, 5, 8 and 11 (Table III) prepared from cod-liver oil extracted with an alcoholic solution of hydrochloric acid; series 19, 22, 25 and 28 correspond to samples 3, 6, 9 and 12 (Table III) prepared from cod-liver oil extracted with aqueous hydrochloric acid. Similarly series 17, 18 and 19 represent the most unsaturated fatty acids, and series 26, 27 and 28 represent the more highly saturated acids separated from the variously treated oils. Intermediate series represent intermediate degrees of unsaturation.

Satisfactory explanation cannot be offered for the lack of results in series 18 and 22. It is clearly evident that the injections were made properly because the onset of the physiological reaction can be seen in both series and in both ears the left treated with the standard and the right treated with the test sample. Idiosyncrasies in the animals may be the cause.



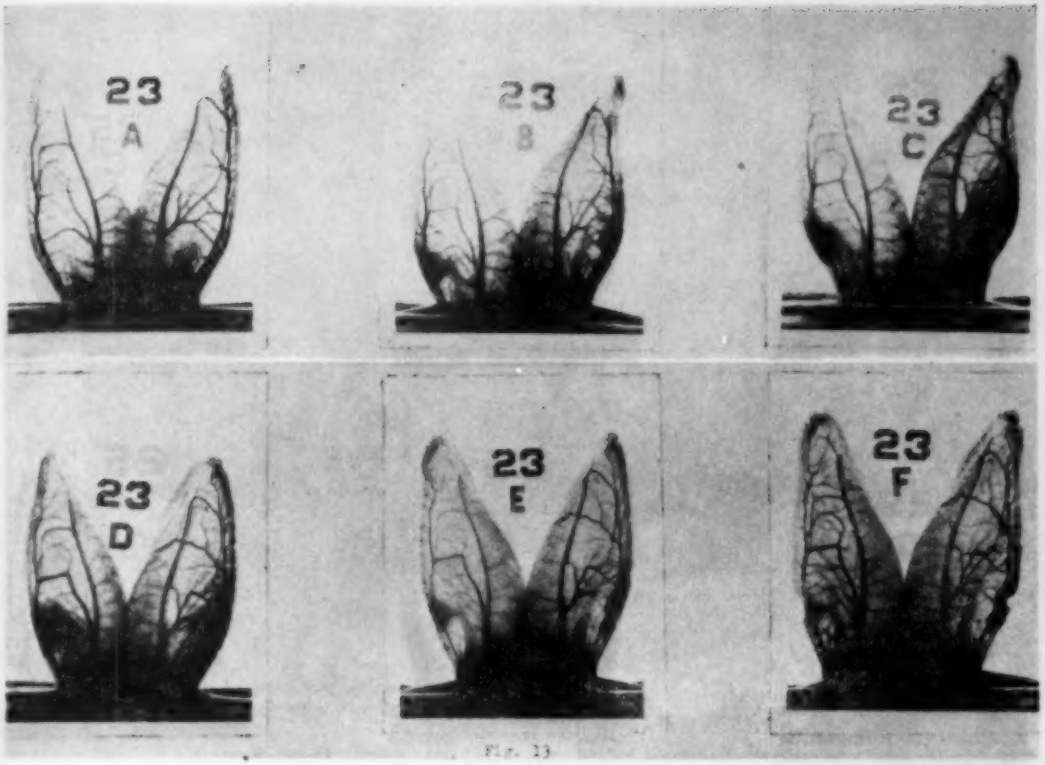
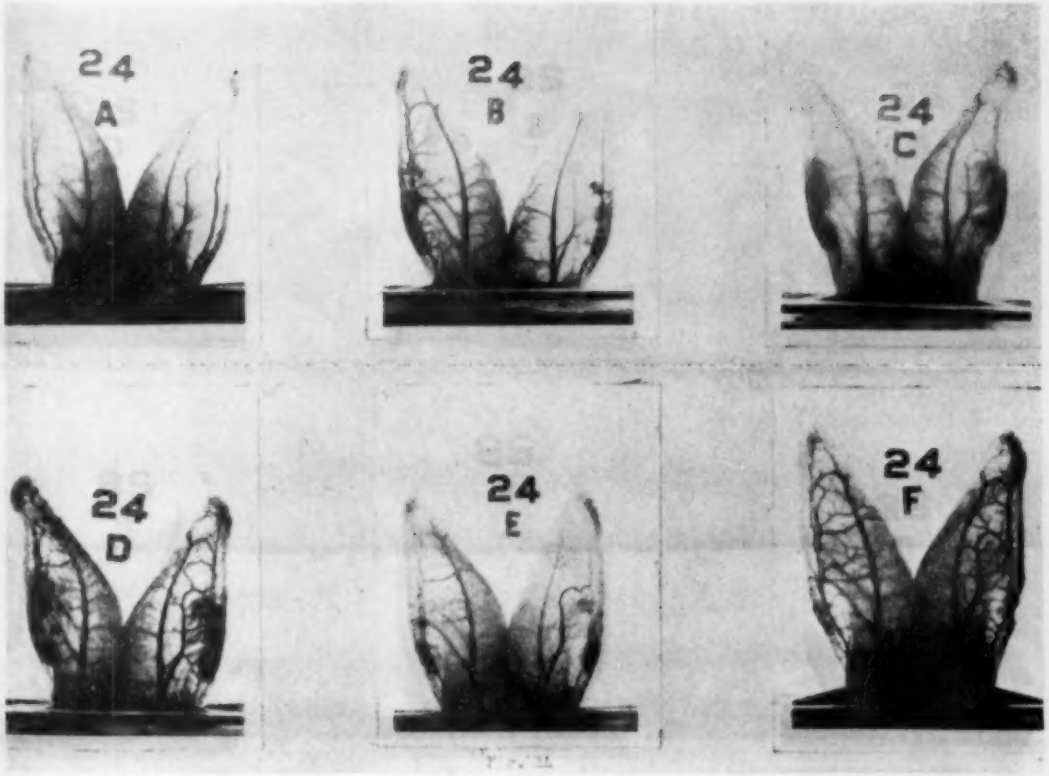


FIG. 23



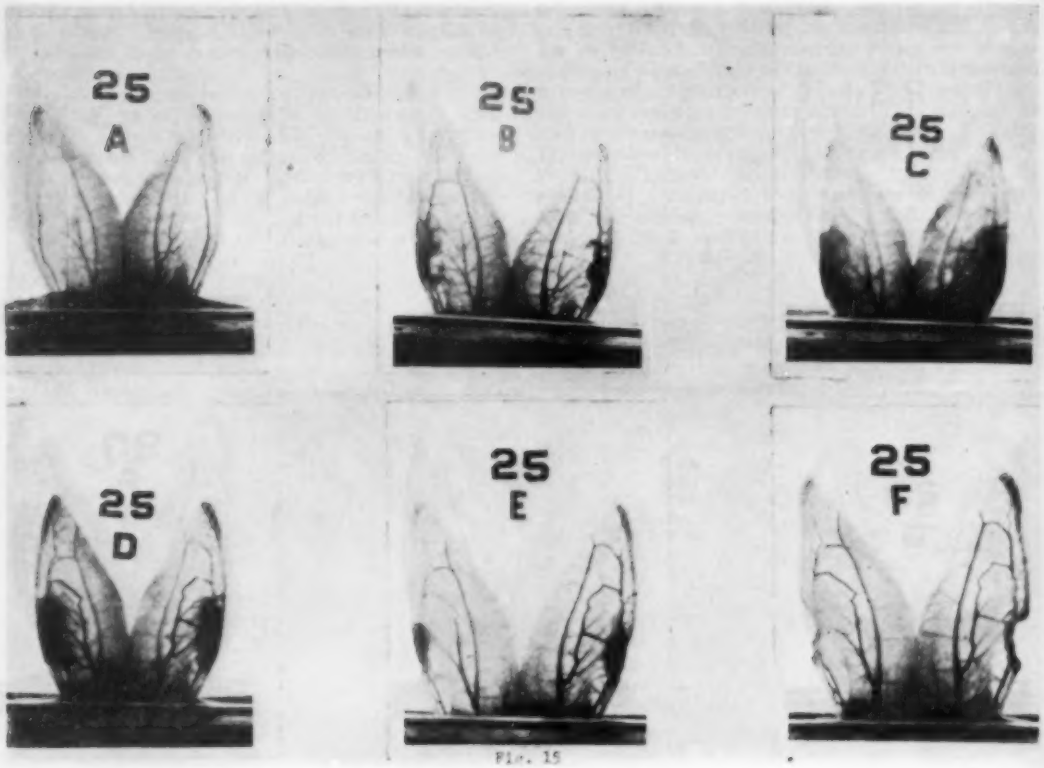


Fig. 25

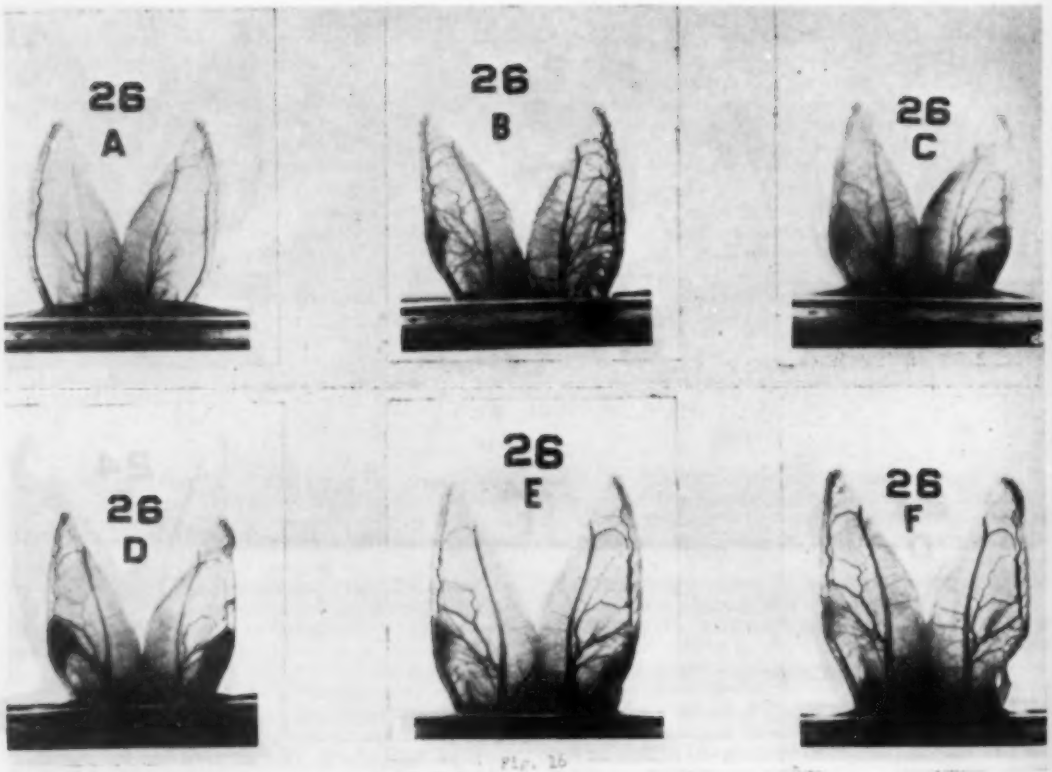


Fig. 26

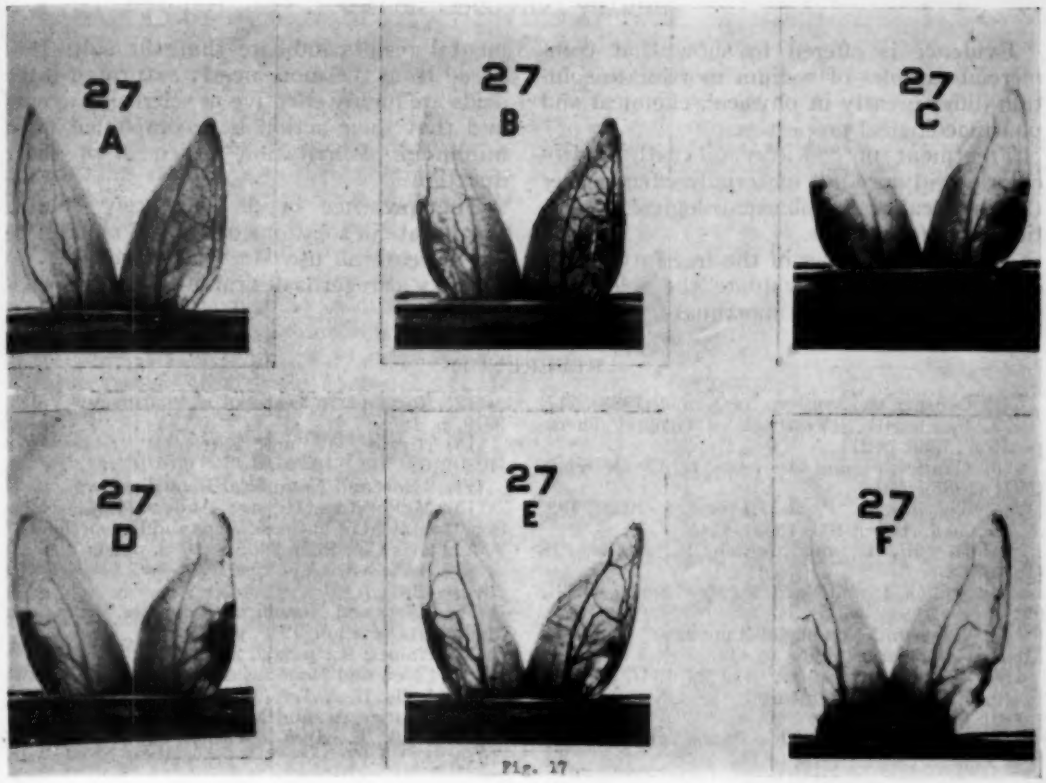


FIG. 27

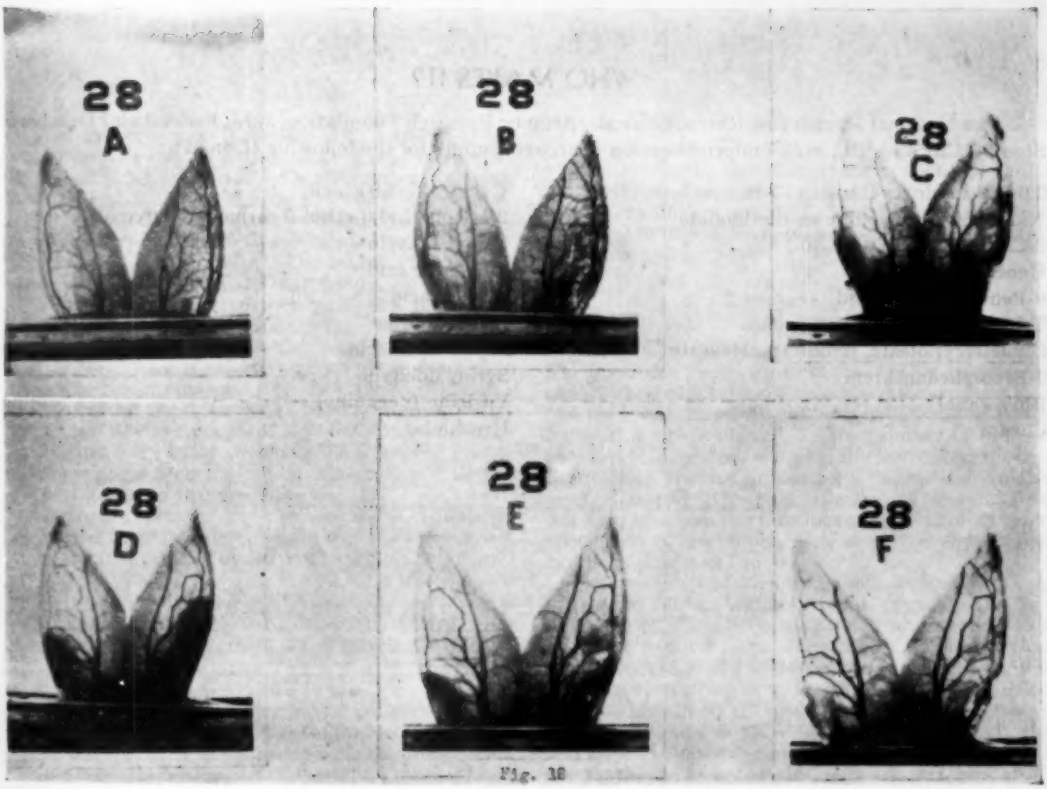


FIG. 28

SUMMARY AND CONCLUSIONS

Evidence is offered to show that commercial samples of sodium morrhuate solution differ greatly in physical, chemical and pharmacological properties.

Treatment of cod-liver oil with hydrochloric acid does not materially effect either the chemical or the pharmacological properties of the fatty acids.

The iodine number of the free fatty acids cannot be used to evaluate the sclerosing properties of sodium morrhuate. Experimental results indicate that the salts prepared from the more nearly saturated fatty acids are highly effective as sclerosing agents and that their action is accompanied by a minimum of irritation which is of short duration.

The presence of a completely soluble precipitate in solutions of sodium morrhuate for parenteral use does not indicate inferiority as regards desirable sclerosing properties.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

2,6,7-Trihydroxy-9-methyl-3-isoxanthone
Nicotine dimethiodide or diethiodide
2-Chlorfluorene (100 Gm.)
Heneicosanoic acid
n-Pentadecanoic acid
Tricosanoic acid
Cholesteryl oleate, palmitate, stearate
9-Bromphenanthrene
Bhilawanol
Cardol
Cyclogallipharic acid
2,4-Dimethyl-3-ethyl-5-carbethoxy pyrrole
Etioporphyrin
Ginkgolic acid
Hyenic acid
Hydroquinine
Psyllosteric acid
Syringaldehyde
Vasicine (peganine)
Urushiol

A Histological Study of *Eriodictyon californicum* II*

By L. David Hiner† and Pauline A. Miller‡

At the 1941 meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION convention in Detroit, a paper entitled "A Histological Study of *Eriodictyon californicum* I," was presented by Hiner and Merrill (1), which contained two definite recommendations for U. S. P. monographic changes for this drug. One of these recommendations which described rosettes of calcium oxalate in the palisade tissue was accepted for U. S. P. XII (2), but the second dealing with the multicellular glandular trichomes was rejected pending additional investigation. The diagnostic histological features noted in the preliminary study were so conspicuous even in the free-hand sections, that it suggested the possibility that a detailed study of the drug never had been made, for otherwise it would have been noted and recorded before that late date. The present study was undertaken as a cooperative and extending piece of investigation to discover and describe more effectively the tissue structures of Eriodictyon U. S. P. XII. Prime interest was centered on the glandular trichomes, and photomicrographs were used in order to represent more accurately the tissues as they appeared in their sections.

EXPERIMENTAL

The leaves were prepared for sectioning by maceration in chloral hydrate solution as suggested in the original paper (1). Concentrations ranging from 5 Gm. of chloral hydrate to 10 cc. of distilled water, to 25 Gm. per 10 cc. were used. The best results were obtained with a solution prepared from 20 Gm. in 10 cc. of water. The leaves were macerated for at least twenty-four hours, rinsed with fresh solution, and were then sectioned for microscopic study. The sections were mounted in a chloral hydrate solution of the same concentration, and in all, leaves selected at random from five commercial samples were used in the investigation.

Typical of the identifying tissue characteristics noted for all the samples of Eriodictyon leaves used in this work are those shown in the following photomicrographs which are all in agreement with the present monographic descriptions.

Microphotography of the glandular trichomes presented considerable difficulty due to the compact cellular arrangement of the multicellular heads and the peculiar wrinkled appearance of the epidermal cells. However, these characteristics themselves are of considerable diagnostic value, and thus become worthy of note in this study (see Figs. 3 and 4).

Fig. 1.—Deep seated glandular trichome with multicellular head of the upper epidermis, and rosette crystals of calcium oxalate perpendicularly arranged in the palisade region. Note depth of the epidermal cells.

It was less difficult to obtain good specimens of the glandular heads bearing the smaller number of cells. They cleared more easily and showed distinct cell wall structures which were easily brought into focus in a single plane. As the number of cells in the head increased, so did the difficulty of distinguishing the individual cells mount due to the intercellular interference. Persistent searching of the field was necessary to locate good photographic specimens of trichomes with even the eight-celled heads as shown in Fig. 6.

The search to locate a trichome for photographing with head cells in excess of eight, disclosed the fact that not all Eriodictyon samples would yield this type of glandular hair. Likewise, it was found that not all leaves of the same sample in which they did occur bore trichomes with more than eight-celled heads. However, there did not appear to be any means of macroscopic differentiation between the leaves, and they all conformed to the U. S. P. XII description microscopically, *with the exception of the*

* Received Jan. 29, 1944, from the Pharmacognosy Research Laboratory, The Ohio State University, College of Pharmacy. Presented to the Scientific Section of the A. PH. A., 1943.

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‡ Bachelor of Science in Pharmacy, June, 1943, Ohio State University.

Fig. 2.—A surface section view of the nearly round stomata of the lower epidermis. Loose palisade cells from the upper portion of the leaf are also shown by the picture. These stomata are much more clearly shown than are those indicated in previous work.

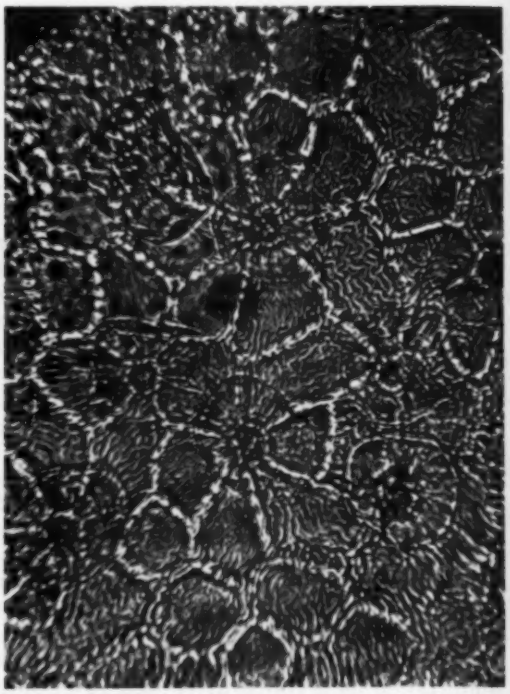

Fig. 3.—Surface view of the wrinkled upper epidermis bearing the glandular trichomes. The heavy walls of the six and seven epidermal cells around the stalks of the trichomes can be seen.

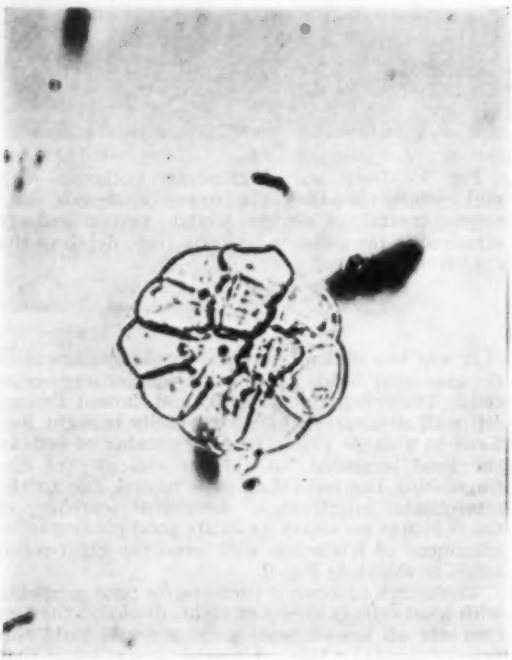

Fig. 4.—The compact cellular arrangement is well shown in this isolated trichome. By changing the depth of focus, 12 distinct head cells could be counted exclusive of the two celled stalk which supported it.

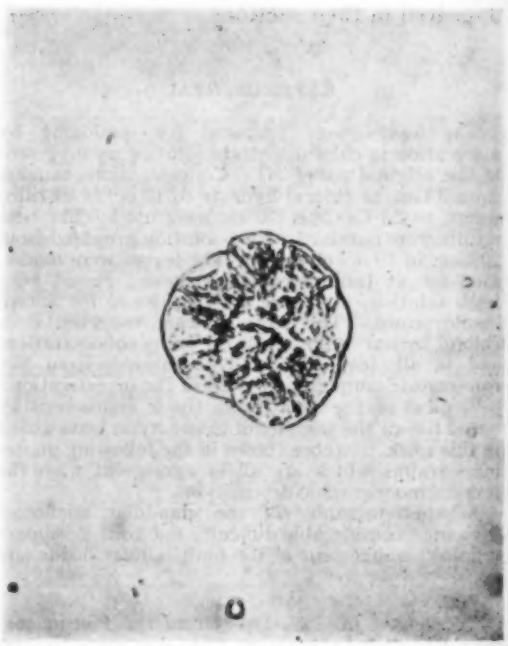

Fig. 5.—Resinous material within the head cell also offered obstacles to photographing. Even after maceration for prolonged periods of time the trichomes failed to clear sufficiently to clearly exhibit their cellular structure.

Fig. 6.—Predominating in number are the typical eight-celled heads which the present United States Pharmacopœia XII recognizes as the maximum number found to date in connection with this subject.

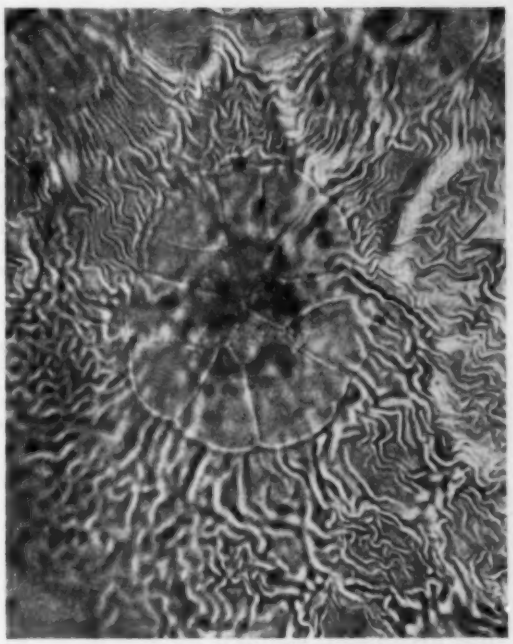

Fig. 7.—Eleven distinct cells can be counted all in one plane of focus. A twelfth cell was faintly visible at the lower right, dividing the largest cell of the group. The characteristic epidermal striations, and the outline of the two-celled stalk can also be seen.

Fig. 8.—The isolated trichome from its side view demonstrates the single row of head cells which made photographing of the surface view possible. This precluded the possiblity of intercellular interference. The two-celled stalk is also visible along with the outline of seven of the head cells.

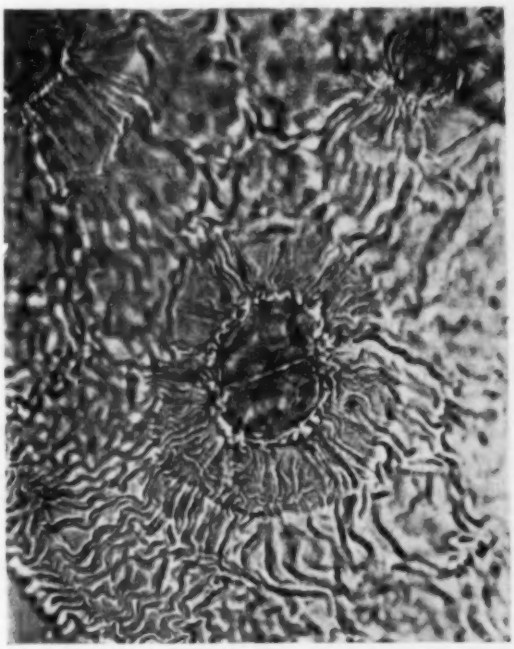

Fig. 9.—To further preclude any question of authenticity of the multicellular head, the focus was changed to bring into full view the two-celled stalk. These are at the level of the epidermis, and only the outline of the head cells as shown in Fig. 7 remain.

trichrome structures. Figures 7, 8 and 9 illustrate this point.

To test out the findings and observations of the ordinary microanalyst, leaves from the sample of Eriodictyon yielding the above figures were given to a group of twenty senior pharmacognosy students with instruction for them to surface section the upper epidermis, mount in chloral hydrate solution, and make counts of the numbers of cells in the heads of the glandular trichomes. No other information was supplied, not even the name of the drug. Each student worked with but one leaf selected at random from the chloral hydrate solution in which the leaves were prepared for this histological study.

From the above results it is obvious that the ordinary microanalyst will observe trichomes in Eriodictyon with head cells in excess of eight, the maximum number cited by the description in United States Pharmacopœia XII. Some students had samples which never showed eight cells, while others easily observed ten or more. Student G later explained that he counted both head and stalk cells in obtaining his consistently high numbers, so his figures were not computed in the final totals. It is easily seen that "*up to eight-celled glandular heads predominate*," and when stated that way, it leaves open the inference that cells in excess of that number may be found.

TABLE I

Student	Number of Head Cells												
	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	2	18	11	8	7	3	2	1	..	..	..	..
B	..	..	5	3	8	6	6	..	..	..	..	..	..
C	..	..	16	11	10	8	5	3	..	..	..	..	..
D	..	..	5	4	3	5	..	..	..	..	..	..	..
E	..	..	3	2	5	4	3	..	..	..	..	..	..
F	..	..	3	1	3	11	23	4	3	1	1	1	..
G[a]	7	7	8	7	7	8	8	8	8	8	8	8	7
H	..	..	6	2	10	8	12	3	2	1	..	..	..
I	..	..	9	1	8	11	26	8	1	..	1	..	..
J	..	..	2	3	11	21	29	4	..	..	..	..	..
K	..	..	2	10	8	5	7	..	..	..	..	..	..
L	2	1	6	6	3	1	8	5	1	..	..	..	..
M	..	..	13	5	5	7	26	..	2	..	..	..	..
N	..	1	4	3	4	4	2	3	1	..	..	..	..
O	..	..	3	1	3	4	17	..	..	..	..	..	..
P	..	..	6	7	6	8	25	3	1	..	..	2	..
Q	..	..	3	4	4	9	16	1	..	..	..	..	..
R	1	1	1	1	..	..	9	..	1	..	..	..	..
S	..	..	3	10	6	2	..	..	..	..	..	..	..
T	..	..	13	7	7	17	24	..	1	..	..	..	..
Totals	4	5	121	92	112	138	241	36	14	2	2	3	0

[a] Student G figures not computed in the totals.

SUMMARY

Glandular trichomes of Eriodictyon were found and photographed containing head cells in excess of eight, the maximum number now cited in the U. S. P. XII.

Samples of leaves bearing these type hairs were sectioned and found to meet all U. S. P. XII description requirements for histological structures thereby establishing the drug's identity. (See figures.)

CONCLUSIONS

It is suggested that the U. S. P. XII monograph which in part reads; "and with numerous deep-seated glandular hairs, possessing a 1- to 3-celled stalk and up to 8-celled glandular heads. . .," be revised to read, "and with numerous deep-seated glandular hairs, possessing a 1- to 3-celled stalk and multicellular glandular heads with up to eight or more cells."

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Dissociation Constants of Slightly Soluble Pharmaceuticals*

By J. Richard Stockton and C. R. Johnson

Investigations by Albert and Goldacre (1), Bell and Roblin (2) and Kumler and Daniels (3) have re-emphasized the significance of relations between drug action and physicochemical properties, especially dissociation constants. Although the determination of dissociation constants of water-soluble weak electrolytes is not particularly troublesome, many pharmaceuticals are so slightly soluble in water that accurate determination of their dissociation constants with aqueous solutions is difficult or impossible. However, these substances are usually soluble in alcohol or other inert organic solvents miscible in all proportions with water, or in solvent mixtures which tolerate considerable water without forming a second phase. Titrations of slightly soluble weak electrolytes in diluted alcohols have been used to evaluate these constants; but the values obtained in this way are not strictly comparable to those measured with aqueous solutions. A simple and reproducible method for determining dissociation constants of slightly soluble weak electrolytes (pK = 4 to 10) from measurements made with solutions in solvent mixtures is described below. The only special instrument required is a pH meter.

The method consists in (*a*) establishing the volume or weight of a standard base, acid or salt equivalent to an accurately measured amount of the pure substance, *e. g.*, by a potentiometric titration in which the neutralization point is found by the "second differential method" (4), or by careful purification and weighing of the substance itself and one of its suitable salts; (*b*) preparing three or four buffer solutions at the half neutralization point, containing different proportions of the organic solvent; (*c*) measuring the apparent pH (as shown by the pH meter) of each of the buffers; and (*d*) plotting pH reading against solvent concentration and determining by graphical extrapolation to zero concentration of the organic solvent the pH of the hypothetical aqueous buffer solution for which the pH is numerically equal to $p\mathrm{K}_a$ (weak acid) or the pOH equal to $p\mathrm{K}_b$ (weak base). The ionic strength of the buffers should be small enough so that the activity coefficients involved approach unity. If very high precision is desired, the pK values may be measured with sets of buffers of varying ionic strength and an additional extrapolation made.

It is characteristic of the precise "second differential method" sometimes used in step (*a*) that the volume of standard acid or base found to be equivalent to a measured amount of the substance is independent of the concentration of the organic solvent or total amount of mixed solvent within reasonable limits. The fact that the pH at the equivalence point is not independent of these is immaterial. If the substance is too weak an electrolyte for a direct titration, a suitable salt may be prepared, and step (*a*) may be carried out by a displacement titration. Alternatively, weighed portions of the pure substance and its salt may be used to make the buffers.

In steps (*b*), (*c*) and (*d*), so long as the pH meter is calibrated correctly for aqueous solutions, it is immaterial whether the capacity of the buffer or the pH values shown by the meter are affected by the organic solvent; any error is removed in the extrapolation, and no other solvent correction is necessary. In fact, while the direct measurement of the pK in any mixed solvent in which the substance actually dissolves is impossible, the accurate estimation of the pK for the substance in pure water is easily accomplished by extrapolation.

To illustrate the application of the method to a specific case, the procedure and data used in measuring the acid dissociation constant of sulfathiazole are given in full.

EXPERIMENTAL

Materials.—A central fraction was taken from U. S. P. sulfathiazole by crystallization and dried in vacuum in an Abderhalden tube; the melting point was 201.8° to 202.2° C. A 1.109 *N* sodium hydroxide solution was prepared from saturated carbonate-free solution and standardized against 1.000 *N* acid prepared from constant boiling hydrochloric acid. Distilled water was boiled to remove carbon dioxide; commercial absolute alcohol was distilled over sodium hydroxide.

Procedure.—A 1.000-Gm. sample of the sulfathiazole was dissolved in a mixture of 100 cc. each of the prepared alcohol and water. The solution was titrated potentiometrically with a calibrated Beckman Model G pH meter, the alkali being added in

* Received March 9, 1944, from the College of Pharmacy and Department of Chemistry, University of Texas, Austin, Texas.

0.040-cc. increments over the equivalence point region. The original and calculated data used in finding the neutralization point are given in Table I. The volume of alkali added at the neutralization point, 3.511 cc., was found by plotting the second derivative *vs.* volume of reagent added and locating graphically the point at which the former changes from positive to negative. An added check was provided here by calculating from the data the percentage purity of the sulfathiazole (mol. wt. 255.3), the value found being 99.5.

TABLE I.—END POINT OF TITRATION: 1.000 GM. SULFATHIAZOLE *vs.* 1.109 *N* NaOH

Cc. Base	*p*H	Δ *p*H/Δ Cc.	Δ^2 *p*H/Δ cc.^{2}
3.360	9.68	...	...
3.380	...	3.5	...
3.400	9.82	...	+ 20
3.420	...	4.3	...
3.440	9.99	...	+105
3.460	...	8.5	...
3.480	10.33	...	+100
3.500	...	12.5	...
3.520	10.83	...	− 30
3.540	...	11.3	...
3.560	11.28	...	−138
3.580	...	5.8	...
3.600	11.51	...	− 33
3.620	...	4.5	...
3.640	11.69	...	...

TABLE II.—*p*H OF MIXED SOLVENT BUFFERS

Alcohol, %	*p*H
20	7.66
40	8.09
60	8.47
80	8.89

The amounts of sulfathiazole and sodium hydroxide solution required for the buffers were then found by proportion. Four 0.5696-Gm. samples of sulfathiazole were dissolved in 200-cc. volumetric flasks containing, respectively, 40, 80, 120 and 160 cc. of absolute alcohol. To each flask 1.000 cc. of 1.109 *N* sodium hydroxide solution was added, and the contents were diluted to 200 cc. with carbon dioxide-free water. The apparent *p*H values of these buffers were then determined at 25° C. (Table II).

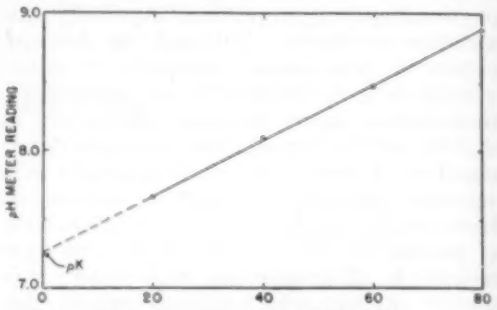

Fig. 1.—Extrapolation to determine *p*K.

A graph (Fig. 1) was then prepared, in which meter readings were plotted against volume percentage of alcohol. The *p*H for zero per cent alcohol, that is, the pK_a of the sulfathiazole in pure water, was found by extrapolation to be 7.26. Another measurement made with a different sample of sulfathiazole (m. 201.8–202.1) and a Beckman Model M *p*H meter gave the value 7.27. These results may be compared with the values of 7.6 obtained by Nielsen and Wolffbrandt (5) and 7.12 found by Bell and Roblin (2).

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